A thesis entitled

ORGANIC GEOCHEMISTRY OF THE MARL SLATE AND OTHER ORGANIC RICH SEDIMENTS

by

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Submitted to the University of Newcastle upon Tyne
for the degree of
DOCTOR OF PHILOSOPHY

Department of Geology

January 1972

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr. A.G. Douglas and Dr. D.G. Murchison for their continued help throughout the course of this postgraduate study. I am indebted also to Dr. B.S. Cooper for the discussions of various topics that have arisen during the duration of this study.

I would like to thank all the members of the Organic Geochemistry Unit for their help, particularly Mrs. A. Summerbell for her help in the drawing of diagrams. Finally, I would like to thank my wife for her support and help, and Miss J. Booker, who kindly typed the manuscript.

This work was supported by a grant from the National Environmental Research Council.

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ABSTRACT

Several samples from the Permian Marl Slate of County Durham and their lateral equivalents in Nottinghamshire and beneath the North Sea have been analysed quantitatively and qualitatively for their geolipid content. Results have shown that a series of normal paraffins ranging in carbon number from C_{12} to C_{32} and a series of isoprenoid hydrocarbons C_{15}, C_{16}, C_{18}, C_{19} and C_{20} are present.

Metalloporphyrins were shown to be present in all of these samples other than those from the North Sea area. A homologous series of etio and phyllo type porphyrins chelated with nickel and vanadium were identified in a concentration of 37 parts per million per unit weight of sample.

The presence of fatty acids in some of the samples was shown to be very low with respect for other shales, e.g. Colorado Green River Shale. A series of normal and isoprenoid fatty acids were identified and shown to be similar to the distribution of alkanes.

Hydrogenolysis experiments of Marl Slate kerogen have shown that mild thermal treatment in the presence of hydrogen and catalyst generates hydrocarbons with a distribution similar to that obtained from the soluble lipid fraction of Marl Slate.

The Miocene Bovey Tracey Lignite of Devon has been analysed and shown to contain a homologous series of normal paraffins and alkanoic fatty acids. Isoprenoid cyclic hydrocarbons are present and triterpenoids previously identified in lignites by other workers have been identified and confirmed.

PART I

AN APPROACH TO ORGANIC GEOCHEMISTRY

CHAPTER I

THE OCCURRENCE OF CARBONACEOUS MATERIALS IN THE EARTH'S CRUST

The Earth's crust, which represents less than 1% of the total mass of the Earth, is recognised as a heterogeneous rock assemblage extending to about 10-13 km. beneath oceanic basins, and to approximately 35 km. beneath the continents. Although carbon is only a minor constituent of the crust (the sixteenth most abundant element) it is distributed throughout, occurring in its elemental form and also chemically combined in various organic and inorganic assemblages.

Geochemically, carbon is one of the most important elements within the biosphere due to its association with hydrogen, oxygen, nitrogen and sulphur. The term biosphere was originally introduced by Lamarck to designate that part of the Earth which is capable of sustaining life. This definition necessarily includes both the atmosphere and hydrosphere as well as the crustal surface. Within the biosphere (0.03% of the total mass of the Earth) carbon is associated with living organisms and Borchert[1] has estimated that the total quantity of carbon in living organisms is of the order of 10^{17}g. Hutchinson[2] has calculated the annual production of organic carbon to be $20\pm5\times10^{15}$g. for terrestrial environments and $126\pm82\times10^{15}$g. for marine environments. Although these figures represent an insignificant mass in relation to the Earth's mass of 6.0×10^{27}g., the presence of living organisms throughout geologic history has resulted in the cycling of over forty elements within the lithosphere. Mason[3] has interestingly shown the geochemical significance associated with this "carbon cycle" which was originally developed by Goldschmidt[4]. Assuming that the mass of the biosphere has remained constant during the last 500 million years, Mason[3] has calculated that the total mass of matter that has been cycled through the biosphere is comparable

to the total mass of the Earth.

During and prior to this period of time organisms, completing their life span, have supplied a continuous "rain" of organic material on to the surface of the lithosphere. The incorporation of this organic material into geological strata and their alterations by physical and chemical interactions, is contained within the discipline of organic geochemistry. The amount of organic matter distributed throughout sedimentary strata, in the form of natural gas, petroleum, coal, tar sands and oil shales etc. has been estimated to be 2.8×10^{21}g. This figure represents only a minute fraction of the total organic material that has been cycled through the lithosphere. However, a comprehensive study of organic geochemistry would not be possible if this recycling process went to completion. Thus sediments containing indigenous organic material i.e. syngenetic with the deposition of the sediment, are represented from every era of geological history spanning over three thousand million years. Unfortunately the period of time between 3.2×10^{9} years ago and the formation of the Earth, about 4.7×10^{9} years ago, was a time of large scale crustal melting[3]. This melting has destroyed, or severely altered the organic matter associated with rock formations of this period. The ubiquity of organic molecules during this and even earlier periods may be deduced from the recent findings of simple organic molecules in interstellar space. This and a seemingly unambiguous identification of racaemic mixtures of amino acids in a recently fallen carbonaceous chondrite[5] has extended the discipline of organic geochemistry both in space and time.

CHAPTER II

THE DISTRIBUTION OF ORGANIC MATERIAL IN MARINE AND NON-MARINE SEDIMENTS

Organic material is found in sedimentary environments of all ages, from recent sediments to those formed in the early Precambrian period e.g. the Onverwacht Series of S. Africa (3.2×10^9 years). A biogenic origin of petroleums and "petroleum-like" organic compounds found in sedimentary rocks is now widely accepted although Robinson[6] has proposed a duplex theory which encompases both biogenic and abiogenic origins. A biogenic origin of the amorphous insoluble organic material, kerogen, which is found in sediments deposited during the Precambrian period is discussed in a recent review by Schopf[7].

The organic matter distributed throughout the lithosphere, estimated at about 2.8×10^{21}g, has been derived almost entirely through burial of organic debris in marine environments. This is not surprising since the bulk of the annual production of organic carbon in the biosphere (ca 75%) is restricted to the marine environment. Also, the degradation products of terrestrially derived organic carbon are carried to the oceans, recycled in the marine food chain or deposited in association with clay minerals as colloids. The annual marine burial of organic material has been continued for over 3.0×10^9 years whilst it is only comparitively recently that flora and fauna have adapted to the terrestrial environment. It is only during restricted intervals of time that coal-producing forests are established. The total amount of fossil coal and peat accumulations is of the order of 10^{18}g, almost insignificant in amount when compared with the value for the lithosphere[3], Table 1.

Since the evolution of the prokaryotic cell to the eukaryotic cell has resulted in the development of organised cell structures showing

TABLE 1. DISTRIBUTION OF CARBON IN THE LITHOSPHERE, HYDROSPHERE AND ATMOSPHERE*

CARBON CONTENT OF EARTH SHELLS IN ORGANIC AND INORGANIC FORMS /	MASS OF CARBON (g)
C_I atmosphere	4.0×10^{17}
C_I hydrosphere	2.8×10^{19}
C_I lithosphere	1.9×10^{22}
C_I limestones	7.2×10^{21}
C_I limestones + shales	9.6×10^{21}
C_I igneous + metamorphic rocks	9.5×10^{21}
C_O biosphere	$2.0 - 2.8 \times 10^{17}$
C_O extractable fossil fuels	4.0×10^{18}
C_O lithosphere	2.8×10^{21}
C_{I+O} TOTAL IN EARTH SHELLS total in earth shells	2.2×10^{22}
Cf. MASS OF ATMOSPHERE	5.0×10^{21}
MASS OF HYDROSPHERE	1.4×10^{24}
MASS OF LITHOSPHERE	2.4×10^{25}
MASS OF EARTH	6.0×10^{27}

/ Abbreviations used: O = organic, I = inorganic

* Data derived and recalculated from BORCHERT[1], HUTCHINSON[2], MASON[3] and RANKAMA and SAHAMA[8].

more complex organisations than those of the prokaryotes, it is possible to show a relationship between the increasing complexity, i.e. evolution of life processes, and the burial of organic carbon in the lithosphere. The development of heterotrophic organisms is closely associated with the burial of organic material in the lithosphere as this is the only means by which free oxygen can be liberated into the atmosphere. Thus the generalised photosynthetic scheme

$$nCO_2 + nH_2O = (CH_2O)_n + nO_2$$

illustrates that free oxygen is only produced at the expense of removal of "carbohydrate type" material, since, otherwise, re-oxidation would take place. The energy liberated by the radioactive decay of heavy elements in the Earth's mantle is directed in several ways e.g. volcanic activity, mountain building etc. which cause the redistribution of parts of the lithosphere necessary for the organic burial process. External energy sources i.e. solar energy influx, are mainly responsible for climatic effects causing continuous circulation of the atmosphere and oceans. If the Earth's crust were not subjected to orogenic cycles, then it would be difficult to envisage a process which would cause the cycling of organic carbon through deep layers of the lithosphere and consequent accumulation of free oxygen in the atmosphere. Without the interplay of geochemical processes, it is difficult to postulate a mechanism by which organisms could evolve from inefficient anaerobic forms showing few organisational characteristics to present day higher organisms. The burial and distribution of organic carbon in the lithosphere illustrates a basic role in the development of life processes.

PART 2

ORGANIC MATTER IN SEDIMENTARY ENVIRONMENTS, LIPIDS, PORPHYRINS AND KEROGEN IN SEDIMENTS

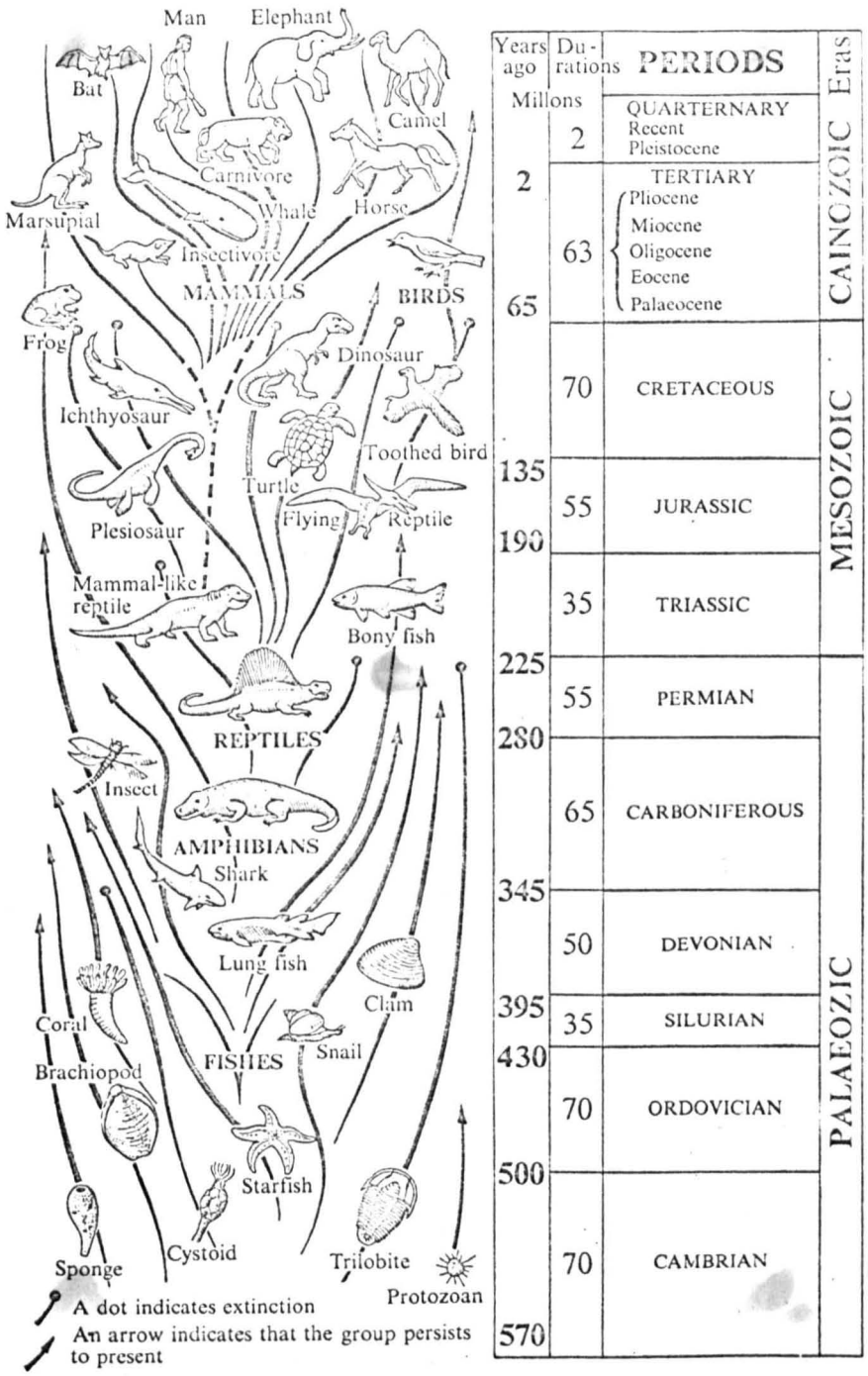
INTRODUCTION

The chemical analysis of sedimentary rocks in the Earth's crust poses several problems. In order to obtain information which can be used for comparative purposes, it is necessary to understand the geological history of the samples that are to be analysed. In the same manner that the history of civilisation has been divided up into periods of increasing chronological age, so also has geological history been divided up into a succession of Periods. Prior to the development of "absolute-age dating" techniques, the geologist had no reliable information about the age of the earth or the length of these Periods. On the basis of palaeontological evidence, i.e. the succession, continuation and evolution of life forms, preserved as imprints or fossils in rocks throughout geological history, it is possible to construct a history of the Earth based upon the evolution of lower organisms to the higher organisms. Rocks which were formed during a Period of geological history constitute a Geological System. The order of the Systems is dependent upon the types of flora or fauna whose morphology has been preserved in the rocks. Thus, on the basis of the fossil evidence associated with a certain sediment, it is possible to assign it to a definite position in geological history.

A geological column is shown in Fig. 1 and it indicates the succession of Periods since the Cambrian Period. The name of each Period has been derived usually from the locality where the strata were first examined. The absolute ages and durations of these Periods are known from radioactive dating techniques, and are shown alongside the column.

The Geological Column contains further subdivisions of strata called Epochs. The rapid evolution and extinction of fauna in the Ordovician System has enabled the System to be divided into seven Epochs, each of which represents a group of strata characterized by a distinct assemblage of graptolites (an extinct order of uncertain affinities).

Figure 1. The geological column.



The evolution of flora and fauna throughout geological history is summarized briefly as follows. During the Precambrian, life forms were either rare or few contained hard parts that could be preserved. The result of this is that it is not possible to correlate Precambrian strata in different areas throughout the world on the basis of palaeontological evidence. The Precambrian was followed by the Cambrian Period during which, from fossil evidence, invertebrates existed which were able to secrete exoskeletons (calcareous or chitinous). Thus, the opportunity for their preservation was increased substantially, and the beginning of the Cambrian Period is marked by the rapid appearance of almost all the invertebrate phylla. These organisms evolved throughout the Ordovician Period and were joined by a number of new organisms. The youngest strata of the Ordovician System also contains vertebrate fossil material, notably fish; these fish followed a rapid evolutionary pattern, such that in the later Devonian Period the former relatives of contemporary fishes were established. Terrestrial plant fossils appeared rather later in time, and until the Silurian Period only algae were represented in the geological record. Vascular plants are first noted in the rocks of the Silurian System, and became more prominent in rocks of the Devonian and Carboniferous Systems. A widespread distribution of fossil ferns is found in the enormous accumulations of coal deposits which were formed during the Carboniferous Period.

The Permian Period is noted for the evidence it contains of new genera e.g. reptiles, and for the extinction of some former genera. During this and the following Triassic, Jurassic and Cretaceous Periods, reptiles diversified, some of them reaching enormous physical proportions during the Jurassic Period. At the end of the Cretaceous Period, many reptiles, e.g. dinosaurs, became extinct.

Mammalian fauna evolved during the Cretaceous Period, reaching their acme in the following Tertiary Period. The evolution of terrestrial flora, notably the angiosperms, first recorded in rocks of the Triassic

System, had by the beginning of the Tertiary Period achieved a contemporary aspect, e.g. sycamore, oak, walnut. During the Oligocene and Miocene Epochs of the Tertiary Period, great deposits of angiosperms and gymnosperms were preserved in Europe as lignites and brown coals.

Due to the apparent absence of fossils in rocks older in age than those represented in the Cambrian System, palaeontologists have not been able to extend the fossil record to the earliest periods of geological history. From the analysis of radioactive isotopes and their degradation products, it has been possible to estimate that the oldest rocks not subjected to subsequent recrystallization are about 3.6×10^9 years in age.

The rock samples which have been studied in this thesis, namely the Marl Slate, Kupferschiefer, Caithness shales and the Bovey Tracey lignite are representatives of sediments formed during the Permian, Devonian and Tertiary Periods respectively. In the shale samples the organic carbon is found dispersed throughout the rock. The organic material is separated by an inorganic mineral assemblage representing more than 90% of the total rock by weight. In these samples, the bulk of the organic carbon is of an amorphous nature, but it is considered to have been derived from algal, bacterial and fish remains with small contributions of terrestrial material.

Rayner[13] has shown that marked similarities occur between the Caithness shales of Scotland and the Green River Shale of the Eocene Epoch, which occupies an area of over 25,000 square miles and outcrops in Colorado, Utah and Wyoming. Both of these sedimentary strata are lacustrine in origin. This author has stated also that some similarities exist between the Caithness shales and the Marl Slate, although it is emphasised that the Marl Slate is of marine origin. The distribution of land surfaces in Europe throughout the Devonian and Permian Periods are shown in Fig. 2 and Fig. 4. During the Devonian Period, strata which

were formed in marine conditions are referred to as Devonian in age, whilst those strata which were formed within the continental areas are referred to as Old Red Sandstone in age. Thus the Caithness sediments which were deposited in a large inland lake, Lake Orcadie, are seen in Fig. 4 to be restricted to the Middle Old Red Sandstone Epoch. During the Lower Old Red Sandstone Epoch the lake was not present and during the Upper Old Red Sandstone Epoch the lateral extent of the lake had diminished greatly. The distribution of land area across what is now Britain is shown in Fig. 2, for the Permian Period. The area marked as the north east cuvette represents a marine area where the Marl Slate and its lateral equivalents in Nottinghamshire were laid down.

Due to the similarities noted by Rayner, and the organic geochemical results discussed in this thesis, it was considered pertinent to compare the Marl Slate, Kupferschiefer and Caithness Shales with each other and to note similarities in their lipid components with those of the Green River Shale (which has been thoroughly studied by other workers). The Bovey Tracey lignite of Oligocene age (ca 26 x 10^6 years), is markedly different from these shales in that it is composed entirely of plant debris of terrestrial origin and consequently is considered in a later chapter.

PART 2A

THE MARL SLATE, KUPFERSCHIEFER AND CAITHNESS SHALES

CHAPTER III

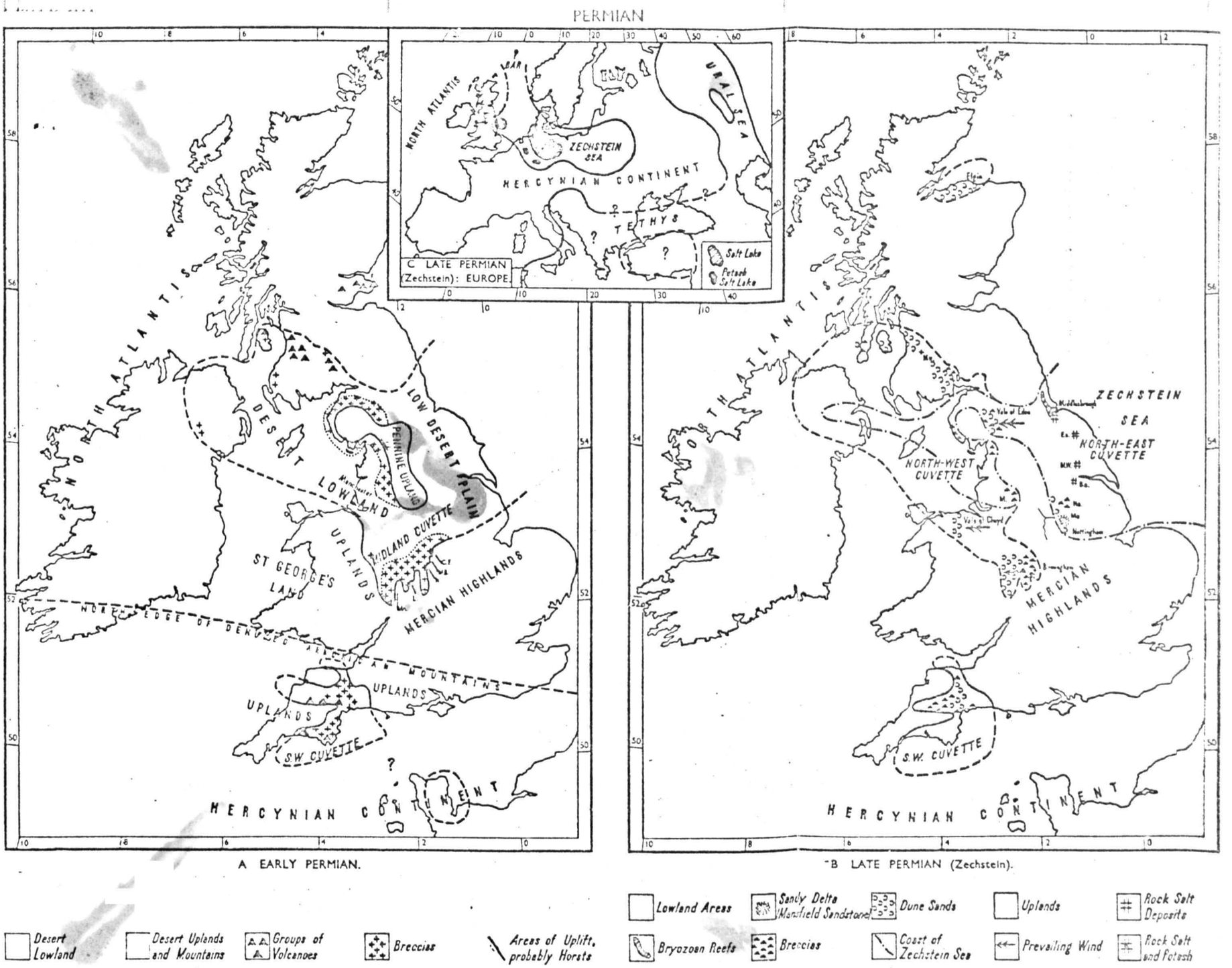
GEOLOGICAL SETTING OF THE MARL SLATE, KUPFERSCHIEFER AND CAITHNESS SHALES

(a) MARL SLATE AND KUPFERSCHIEFER

The 'Marl Slate' of County Durham is a calcitic or dolomitic bituminous siltstone varying in thickness from 0.75 metre to about 4.0 metre and averaging 1.32 metre from over 60 borings[9]. The organic carbon content may constitute up to 11.5% of the rock weight but in the samples analysed the maximum was found to be 7.68%[9].

During the early Permian Period (ca. 280 x 10^6 years ago) the British Isles formed part of a semi-arid continent which was undergoing denudation resulting in peneplanation of the highlands. The north-eastern area of England, extending from Durham southwards to Nottinghamshire, formed a low desert plain into which extensive deposits of dune sands, referred to as the Yellow Sands accumulated. Further to the east a thin conglomerate was deposited, referred to as the Zechstein conglomerate, which separates the Rotliegende and the Kupferschiefer of Germany. It has been suggested that during the late Permian Period (ca 230 x 10^6 years ago), a shallow epi-continental sea (Fig. 2) spread across what is now Germany and the North Sea into northern England to connect with the Boreal Ocean to the north. This sea is believed to have received ocean water from time to time, but subsequently decreased in size owing to evaporation. Evaporite deposits were generally confined to the deeper parts of the basin, whilst red beds, limestones and dolomites were deposited close to the margins of this sea[10,11]. In this environment, under essentially stagnant bottom-water conditions, the preservation of organic material (sapropel) occurred, which today is represented by the kerogen content of the Marl Slate sediment and its lateral equivalents in Nottinghamshire and the North Sea.

Figure 2. Distribution of land surface during the Permian[10].



Hirst and Dunham[9] have studied the petrology and inorganic geochemistry of the Marl Slate of Co. Durham and have reviewed the petrographical evidence for the nature and rate of deposition of this sediment. Petrographic evidence has indicated that brief periods of accumulation of carbonates and clays alternated with periods of deposition of organic matter (sapropel). This organic matter is associated with neither carbonates nor clay, but forms lenticular bands which separate these constituents. Pyrite occurs and is closely associated with organic matter, approximately 95% of the pyrite being enclosed by it. It is believed that the pyrite was formed very early during diagenesis, under the influence of micro-organisms, tentatively identified by Love[12] as belonging to the sulphur bacteria group, Desulphovibrio desulphuricans. The alternation of organic material with either carbonate or clays has been related to an annual climatic cycle which allows a period of deposition of the Marl Slate to be in the order of 17,000 years[9].

In their study of heavy metal concentrations in the Marl Slate, Hirst and Dunham[9] reported an association of Co and Ni with organic carbon. It is believed that these elements, along with Pb, Zn, Cu, Mn and Sr, were carried into the lagoonal sea by fluids released from the hydrothermal mineralisation in progress to the west of, and beneath, the Marl Slate lagoon. Love[12] has suggested that these metals were then adsorbed on organic particles and subsequently converted into sulphides in the later stages of diagenesis.

Smith and Francis[11] have briefly reviewed the fossil flora and fauna of the Marl Slate. In places, great quantities of palaeoniscid fish remains occur in association with plant remains e.g. Ullmania. They have also noted the paucity or absence of bottom fauna which is most probably related to the stagnant water conditions at the bottom of the lagoon. Microfossils have been recognised in the calcite and

dolomite constituents of the sediment by examination of polished samples with ultra-violet light fluorescent microscopy. Tentative identification of these microfossils by the author show that they belong to the Hystrichosphaeridae. Three different morphological forms are apparent and the one shown in the photograph, Fig. 3(b), is tentatively identified as belonging to the genus Michrystridium. Baltisphaeridium, and Veryhachium genera have also been identified*. All specimens were less than 35u in total cross-measurement. A search of the literature available on the hystrichospaeridae has shown that they have only been found in marine sediments*.

Sections at right angles to the bedding of the samples studied (Marl Slate Co. Durham) has revealed a small contribution of terrestrial material to the environment of deposition. This material, probably transported to the Marl Slate lagoon by winds, consists of spores, Fig. 3(a), which appear elliptical due to compression; they fluoresce a bright yellow in ultra violet light. A typical spore is shown in the photograph, Fig. 3(c), where the cell walls appear white.

ORIGIN AND COLLECTION OF SAMPLES

Eight samples were obtained for analysis, seven of them being recovered from borings. The Marl Slate (Downhill) sample was obtained from the Downhill quarry near Sunderland (G.R. 605350). Dr. Pattison (Leeds Geological Survey) supplied the following borehole samples; YFF (1626) from the Mainsforth Colliery No. 5 borehole Co. Durham NZ (30583212); BU (4930) from the Fishburn No. 1 Borehole Co. Durham NZ (36853325); BQ (7276) from the Kneesall borehole, Nottinghamshire SK (7135364380); BK (9825) from the Milton borehole, Nottinghamshire SK (71353 64380); DC (322323) from the Middle Stotfold Borehole Co. Durham NZ (45005 29867). Two borehole samples from the Kupferschiefer, (K-1 and K-2), were donated by Dr. P.E. Kent (Chief Geologist, British Petroleum Ltd.).

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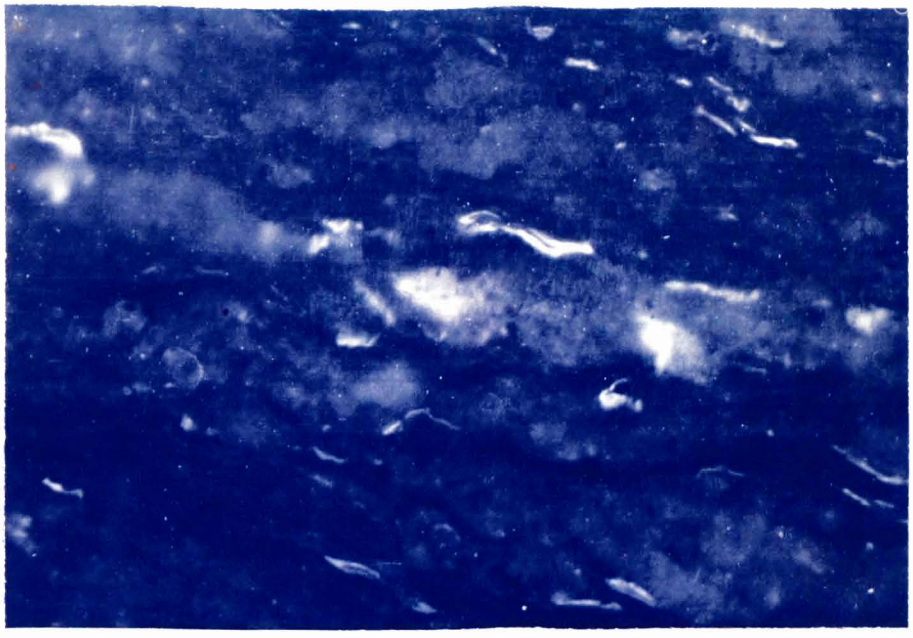
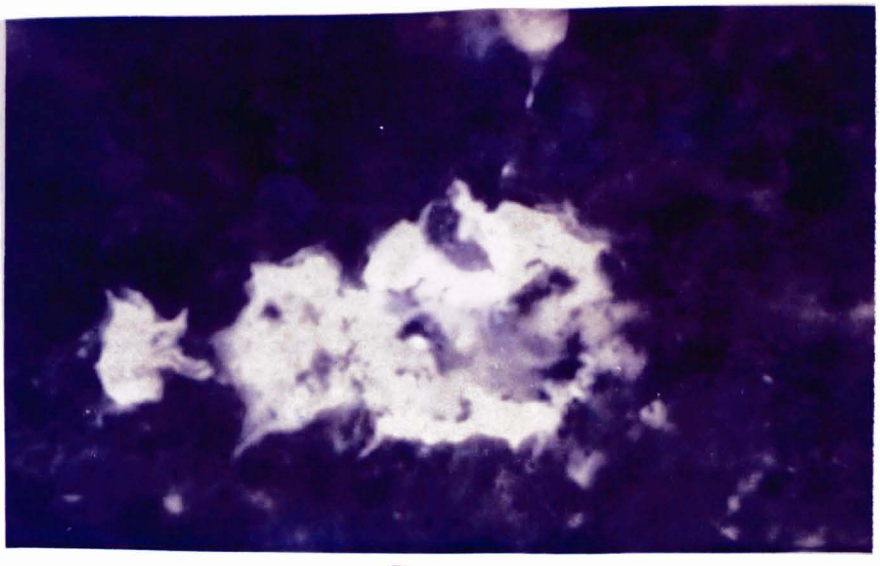
* Personal communication from Dr. W. Sargeant, Department of Geology, University of Nottingham

Figure 3. Fluorescent light microscopy of polished sections of Marl Slate.

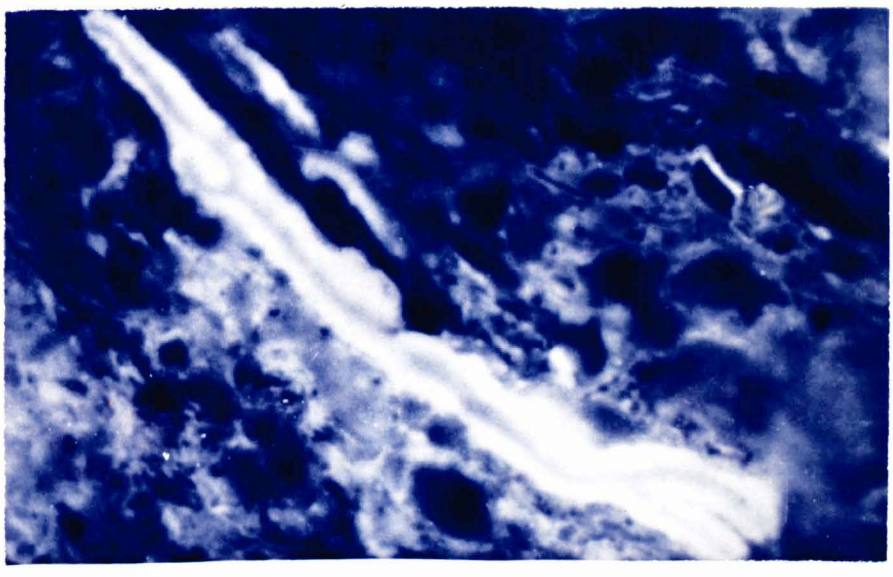


(a)



(b)

30μ.



(c)

Sample K-1 was obtained at a depth of 8,698 ft. The temperature at this horizon was 77-78°C in the location BP 48/6-1.

Sample K-2 was obtained from West Emsland, at a depth of about 10,000 ft (horizon temperature 127°C).

(b) CAITHNESS SHALES

During the Middle Old Red Sandstone epoch (ca. 360 million years ago), some 20,000 feet of sediments were deposited in a large inland freshwater lake, Lake Orcadie, located as shown in Fig. 4. Sedimentation occurred at or near water-level, or subaerially, giving rise to mudstones, sandstones and limestones. Rayner[13] has described the rhythmic deposition of these sediments as occurring in units of between 30 and 70 ft.

The sediments contain well-defined facies with recognisable carbon-rich and clastic layers. In some of the sediments, e.g. in the Achanarras Limestone, well-defined laminae are present which are believed to be due to seasonal or annual variations in the type of material accumulating on the lake floor. Petrological evidence[13] suggests a seasonal sequence of carbonate precipitation followed by plankton blooms in the surface waters, later giving rise to accumulations of organic sapropel. Large numbers of fish remains have been reported, the more important comprising Palaeospondylus, Coccosteus, Homosteus, Pterichthyodes, Dipterus, Osteolepis, Glyptolepis, Cheirolepis and Cheirocanthus[13].

Rayner[13] suggests that the sediments were deposited in conditions very similar to those which prevailed 300 million years later, when the Green River Shales of Colorado, U.S.A. was deposited. Similar features of deposition are found in the Midland Valley of Scotland when oil shales of Lower Carboniferous age were deposited[13].

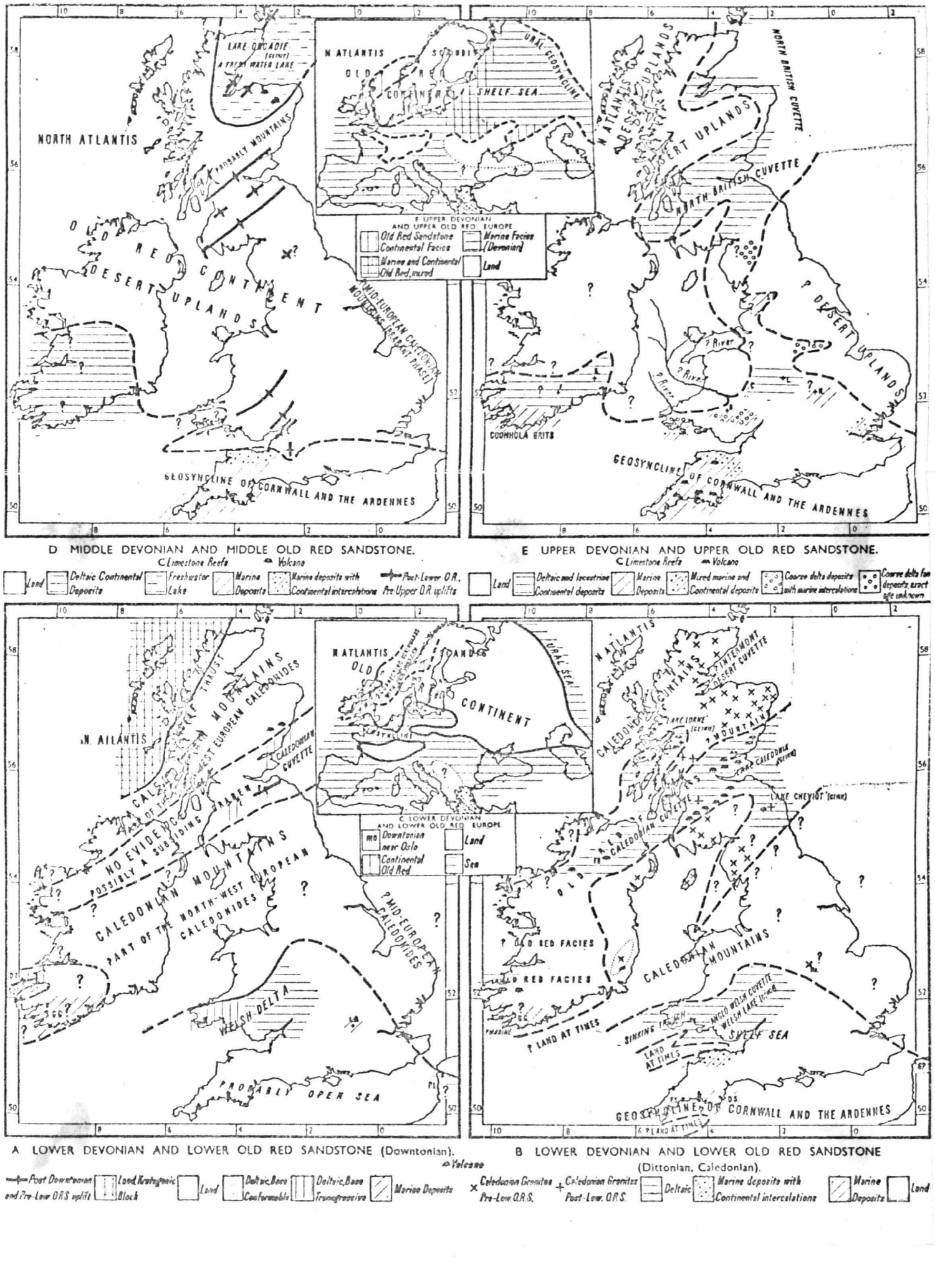


Figure 4. Distribution of land surface during the Devonian[10].

The Middle Old Red Sandstone shales often contain laminations that are rich in organic carbon. Thus a hard, black, bituminous shale occurs in bands on the coast at Barrogill, Ackergill, Keiss, Freswick, and was once quarried inland at Killimster. The black shale is locally called the "black man" and yields hydrocarbons on distillation. This bituminous matter is believed to have originated from algal remains with possible contributions from plant debris[14]. Fluorescent microscopy of the two samples studied in this work showed no visible remains of spores or plant fragments, most probably due to the fact that plants, although established in the terrestrial environment were limited in distribution.

COLLECTION OF SAMPLES

Both of the samples discussed below were collected in an unweathered condition by Mr. N. Donovan of the Geology Department, Newcastle upon Tyne.

Sample A was a black bituminous shale from Wideford Hill, Walliwall Quarry on the Mainland of Orkney about 1 mile west of Kirkwall, in the Rousay Flags (Grid Ref. HY 2433 1010).

Sample B was obtained from a quarry in the Wick Beds of the Wick Flagstone group to the north side of Wick Bay (Grid Ref. ND 3820 5090). The unweathered sample was recovered from a horizon 10 ft above the base of the quarry.

CHAPTER IV

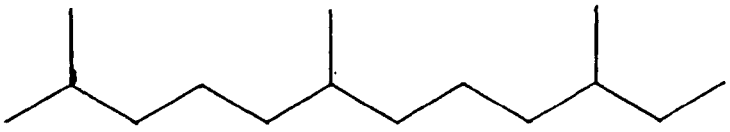
LIPIDS IN THE MARL SLATE, KUPFERSCHIEFER AND CAITHNESS SHALES

(a) INTRODUCTION

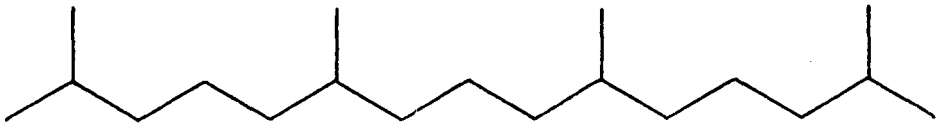
An estimated 10^{17}g of organic carbon is synthesised annually by living organisms[3]. Upon the death of the organism some of this organic material escapes recycling in the food chain of all life processes and falls as organic debris to lower horizons of the biosphere where it may be incorporated with rock forming minerals. It is believed that this organic debris has been altered, in first aerobic and then anaerobic environments, by chemical and microbiological agencies. After prolonged periods of exposure at elevated temperatures saturated hydrocarbons related to original lipid material often remain as predominant constituents of the soluble organic material. Some unusual hydrocarbon deposits are known however, and these may possibly reflect an abiogenic origin or the effect of very high temperatures on biogenically derived organic material[15,16,17].

Most investigations of the organic material preserved in sedimentary formations have been concerned with specific organic compounds occurring in the extracts of sediments. These organic compounds which contain a structural specificity, indicative of a biogenic origin, are generally referred to as biological markers. The saturated hydrocarbons so far found to occur in these sedimentary rocks consist of normal, iso- and anteiso-alkanes, isoprenoid and cycloalkanes. The iso- (2-methyl), anteiso- (3-methyl) and isoprenoid alkanes such as farnesane, pristane, and phytane, whose structures are shown in Fig. 5, are the most commonly identified branched-chain hydrocarbons occurring in sediments and petroleums. They

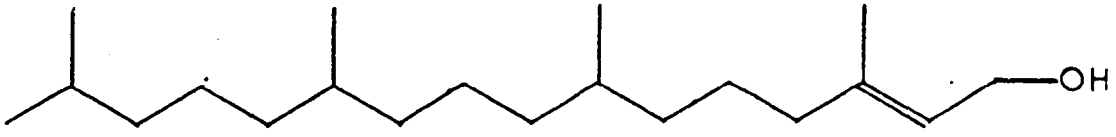
Figure 5



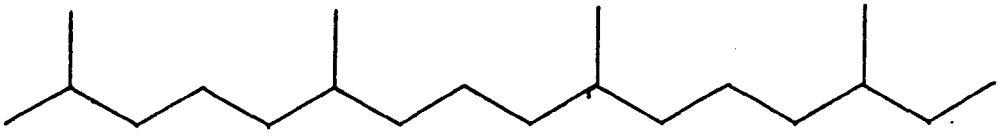
FARNESANE



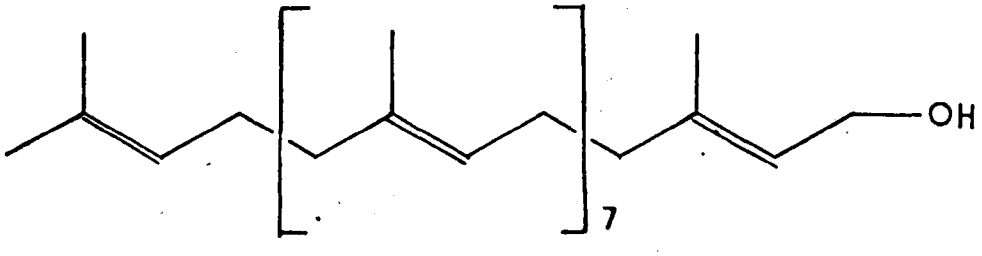
PRISTANE



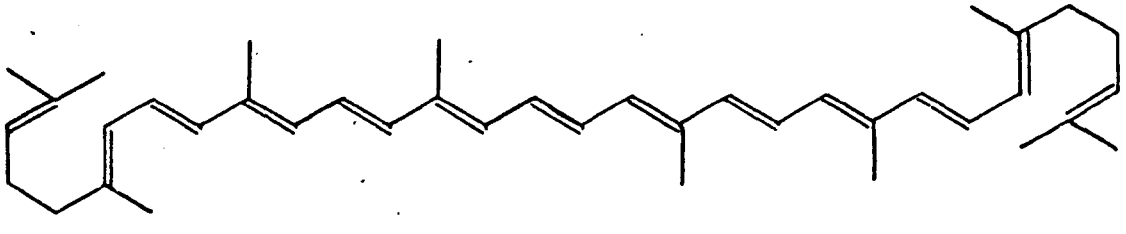
PHYTOL



PHYTANE



SOLANESOL



LYCOPENE

are considered to serve as biological markers, indicating the former presence of living organisms in those environments. Normal alkanes are found extensively in nature, occurring in terrestrial plant and animal lipids[18,19]. They are often found in association with iso- and anteiso-alkanes in plant waxes[20], insect waxes[21], fungi[22] and algae[23]. It has been observed that terrestrial plants have normal alkane distributions which have a maximum in the C_{27} - C_{33} region, whereas more primitive organisms, such as algae and bacteria, often contain alkane distributions which have a maximum between n-C_{15} and n-C_{19}. The carbon-number region at which the normal alkanes are at a maximum has been used as a factor in determining either a marine or terrestrial origin for the organic material that produced a particular petroleum[24].

There is comparatively little literature which deals with the extent to which the lipid-cell constituents and metabolites of marine organisms[25], survive uncharged in the sediment. Some of the organic material that is deposited on the marine floor is utilised in the food chain of other organisms, and the organic debris that is eventually buried is believed to contribute to the nutrient and energy requirements of anaerobic bacteria. The degree to which bacteria alter and contribute to the organic biomass is as yet little understood. Following the work of Trask and co-workers, the American Petroleum Institute launched an investigation into the role played by bacteria in the transformation of organic material to petroleum. The result of this research has been reviewed by Davies[26]. This work demonstrates that the depletion of hydrocarbons in recent sediments is due to oxidative microbial activity, whereas the richest source beds of petroleum contained substantial quantities of hydrogen sulphide, indicative of anaerobic sulphate-reducing bacteria. Cooper and Blumer[27] have reported the presence in recent marine sediments of

iso- and anteiso- fatty acids in the range C_9-C_{21}. These authors conclude that these branched acids may arise from bacterial sources in the sediment. The isolation and identification of branched-chain fatty acids in the genus Bacillus has been reported by Kaneda[28] and by Kates[29]. Recently, more work on the hydrocarbon components of bacteria[30,31] and algae has been published. The sulphate-reducing bacterium, Desulphovibrio desulphuricans[31], which commonly occurs in marine sediments, contains a prominent n-C_{18} alkane, but the greatest percentage of alkanes lies in the range n-C_{25} - n-C_{33}. Since Stone and Zobell[32] noted that some bacteria synthesise paraffinic hydrocarbons, it has become increasingly important to study the hydrocarbon content of bacteria and to determine how they contribute to the marine sedimentary environment. It is known that the normal alkane distributions of most ancient sediments and petroleums have a carbon preference index (CPI)* of about unity in a given homologous series. Various authors have postulated such mechanisms as the decarboxylation of fatty acids to hydrocarbons[33,34], in order to derive CPI values of unity. It is now apparent that bacteria may contain n-alkanes with no odd or even carbon preference[35,36]. Oro et al[37], and Calvin et al[38,39,40,41] are investigating the hydrocarbon content of bacterial cells in order to determine the contribution that they make towards the precursor material from which petroleum is derived. Similarly, Kaneda[42] and Parker et al[43] have reported that bacteria are a prominent source of branched-chain fatty acids, which although uncommon in more specialised organisms, are of common occurrence in some ancient sediments and petroleums. It is quite probable therefore that a detailed survey of the lipid components of bacteria may prove useful in explaining certain general observations in this field.

The C_{15}, C_{16}, C_{18}, C_{19}, C_{20} and C_{21} isoprenoid hydrocarbons are widely distributed throughout ancient sediments and petroleums. They are present

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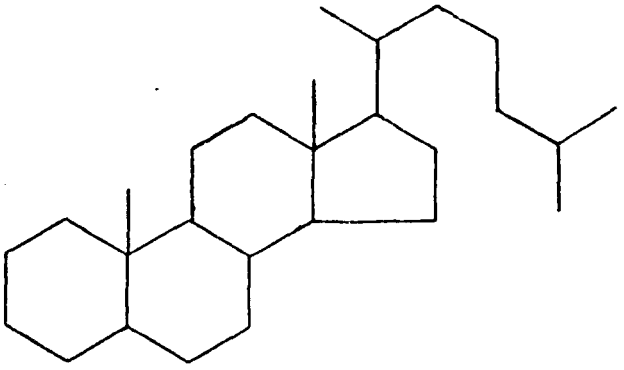
* $CPI = \frac{\sum nC_{odd}}{\sum nC_{even}}$

in varying concentrations in the soluble mineral extracted from oil shales, amounting to some 3.4% of the solvent extracted bitumen of the Colorado Green River shale. This shale represents the most extensively studied ancient sediment as regards its "geolipid" content. Analysis of the palaeontological evidence, and the hydrocarbon content of this shale, has suggested that blue green algae and pollen from terrestrial plants may have contributed to the organic matter in the sediment[44].

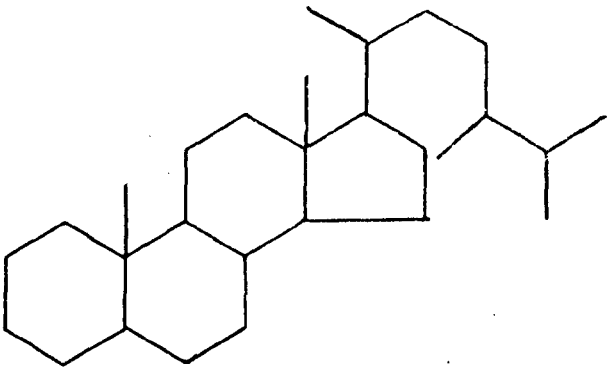
The presence of steranes, triterpanes and tetraterpanes has been established in ancient sediments. Compounds identified include cholestane, ergostane, sitostane and perhydro-β-carotene[45,46] whose structures are shown in Fig. 6. Cummins et al[47] isolated a pentacyclic triterpane from the benzene extract of Green River Shale which was identified by Hills et al as gammacerane (tetrahymane)[48]. This symmetrical and optically active pentacyclic compound is thought to be the simplest triterpane system that may be derived biosynthetically from all chair-coiled squalene. Other triterpanes related to gammacerane have been tentatively identified in the geological environment. These compounds include hopane and moretane as well as the rearranged forms filicane, fernane, adianane and arbcrane[49,50].

The saturated acyclic isoprenoid hydrocarbons are found abundantly in ancient sediments of varying ages, but only the C_{19} isoprenoid hydrocarbon pristane has been reported in the marine environment[51]. Other isoprenoid hydrocarbons are known in the marine environment e.g. squalane, but as yet it has not been detected in ancient sediments and only tentatively in petroleum. A most likely precursor of pristane is the mono-unsaturated diterpenyl alcohol, phytol, which occurs as an ester in some chlorophylls. Pristane has also been found in marine organisms, e.g. sharks[52], copepods[53], marine algae[19], and in a wax found floating in an estuarine environment[54,55]. Bendoraitis[56] has suggested that phytol might be the precursor of the C_{15}, C_{16}, C_{18}

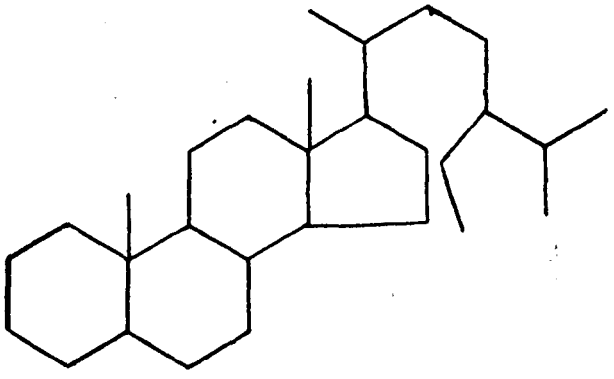
Figure 6



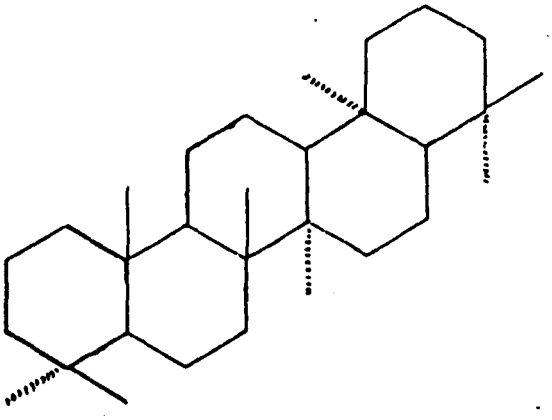
CHOLESTANE



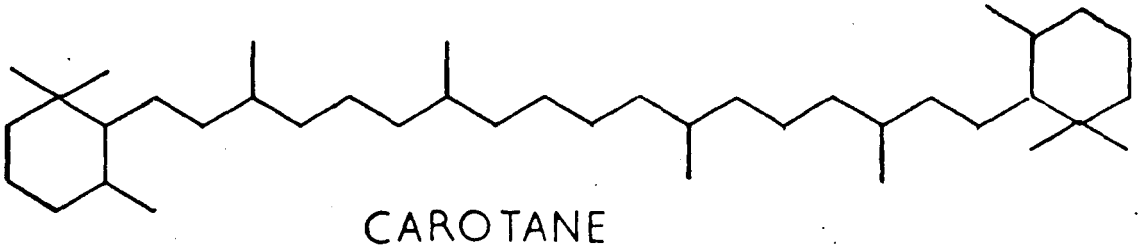
ERGOSTANE



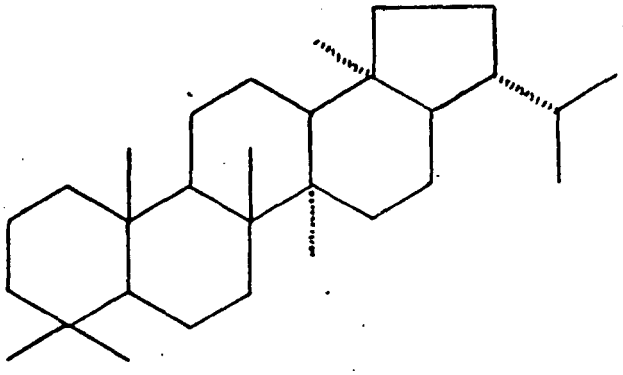
SITOSTANE



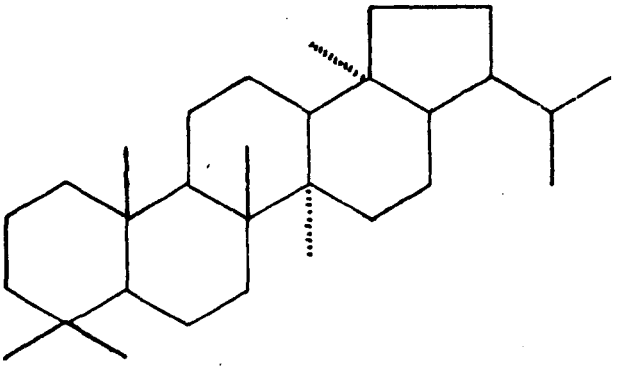
GAMMACERANE



CAROTANE



HOPANE



MORETANE

and C_{19} saturated isoprenoid hydrocarbons found in sediments. The formation of these hydrocarbons would require the cleavage of only one carbon bond, whereas a C_{17} isoprenoid would require that two bonds be broken. Calvin et al[57] have reported the C_{17} isoprenoid in a Devonian shale along with the other C_{16}, C_{18}, C_{19} and C_{21} isoprenoid hydrocarbons. The C_{17} isoprenoid is present at a much lower concentration than the other isoprenoids and it seems to accord with a diagenetic scheme in which phytol is considered to be the precursor of these isoprenoid hydrocarbons. Further work[58] has been carried out to determine whether the C_{21} - C_{25} isoprenoids could be derived from high molecular-weight polyisoprenols, such as solanosol (C_{45}) with a regular head-to-tail isopentene linkage or lycopene (C_{40}) containing a tail-to-tail linkage of the two C_{20} moieties. The structures of these compounds are shown in Fig. 5.

Investigations of recent sediments which represent the modern counterparts of rich oil shales, such as those of the Green River formation, may lead to an understanding of the types of organisms that have contributed towards the formation of petroleum. Calvin et al[39] have identified some of the lipids present in an algal ooze from Mud Lake, Florida. This ooze is believed to represent a system which could eventually provide rich oil shales. The presence of predominantly n-C_{27} and n-C_{29} alkanes suggests that wind-blown pollen has contributed to this ooze. There is little morphological evidence that higher plants have contributed significantly to the hydrocarbons of Mud Lake or the Green River shale, other than pollen and spores which are found in abundance in the Green River Shale and to a lesser extent in the Mud Lake sediment. Although there is a bimodal distribution of n-alkanes in the Green River Shale, with maxima at n-C_{17} and n-C_{29}, there is no evidence for the presence of the n-C_{17} alkane as a major constituent of Mud Lake Sediment. The ooze is constituted almost

exclusively of blue green algae detritus and it appears surprising that the n-C_{17} alkane is missing. The fatty acid content of blue green algae indicates that the dominant acids are in the range C_{16} to C_{18}[40]. Unsaturated fatty acids may rapidly polymerise by a Diels Alder type of reaction[59,60], or very early in the history of the sediment they may become "bound" with other organic material to produce kerogen. Saturated fatty acids are known to be linked to the kerogen by ester bonds in some of these sediments, e.g. Green River Shale. Although the structure and mode of formation of this kerogen is complex, the release of n-alkanes by thermal cracking at elevated temperatures is well established[61,62].

The distribution and structures of fatty acids in sedimentary environments have been examined for several purposes. Initially they were sought since they are major components of living matter and the saturated members belong to the more thermally stable of organic substances. Their occurrence in rocks of all ages, petroleums and petroleum waters[63] suggested that they could be responsible for the formation of normal alkanes[33,34]. Further work showed that the amounts of free fatty acids or esters extractable from ancient sediments and recent sediments are relatively small[64]. The most abundant saturated fatty acids found were stearic (C-18), palmitic (C-16) and myristic (C-14), whilst unsaturated fatty acids were absent, even in recent sediments[63]. Only about 1 part in 1,000 of these acids remains in extractable form after a short period in the sedimentary environment. This disappearance is believed to be due at least partly, to their incorporation into the kerogen. Prolonged exposure to diagenetic processes may release the alkyl chain in the form of long chain hydrocarbons. Although no information is as yet available, this same process may be characteristic of fatty alcohols.

Some naturally-occurring normal and branched fatty acids must survive chemical and biochemical degradation during incorporation into

sediments since they are found in ancient sedimentary rocks. Parker has briefly discussed the distribution of branched acids in ancient sediments[65]. In the recent sediments that have been analysed, the distribution of normal fatty acids is similar to the distribution of naturally-occurring normal fatty acids, even carbon-numbered normal fatty acids being predominant. With increasing age of the sediment, the even/odd ratio of the normal fatty acids decreases and may approach unity.

Abelson and Parker[64], Cooper[63] and Kvenvolden[66] have analysed various recent sediments and have reported this high even/odd predominance. Meinschein and Kenny[67] have also reported this high even prodominance of normal fatty acids in soils, over the range C_{14} to C_{30}. Recently, the presence of C_{16} - C_{24}, even-numbered hydroxy fatty acids, dihydroxy and α - and β -hydroxy acids has been reported from a 5,000 year old lacustrine sediment[68,69]. A substantial amount of work has been reported on the occurrence of fatty acids in the relatively young Green River Formation (ca. 60 million years)[70-76].

Cason and Graham[77] noted the occurrence of the isoprenoid, pristanic and phytanic acids in a Californian petroleum. Following this report the presence of the same isoprenoid acids was reported in Green River Shale[71]. This shale has since been shown to contain other isoprenoid acids, (see Table 36, page 39.) including α - ω dicarboxylic acids[74], iso and anteiso acids, methyl keto-acids and aromatic acids[75]. These acids have been identified as free acids, occurring in the organic soluble extract, as "bound" acids occurring as metal salts in the inorganic matrix and as esters bound to the kerogen. The acids bound as salts have been freed by acid digestion (HCl and HF) and are present to a much greater extent than the free acids. Isoprenoid acids have also been identified in chromic acid oxidation products of the kerogen.

Abelson et al[70] isolated even-numbered fatty acids from the Mahogany Zone of the Green River Formation. Lawlor and Robinson[76]

showed the presence of normal fatty acids ranging from C_{10} to C_{34} with even-numbered homologues predominating. Investigations of the distributions of fatty acids in older sediments show a similar carbon-range distribution, but with a more marked increase of the odd-carbon numbered homologues. Cooper[63] noted a range of normal fatty acids, from C_8 to C_{28}, in a Mississippian (Carboniferous Period) shale which showed only a slight even/odd predominance. However, some workers have reported that the presence of the fatty acids in these samples is restricted to C_{14} to C_{22} but the techniques which were employed would have caused appreciable losses below the C_{14} acids. Recently, Van Hoeven et al[78] have reported the presence of very small concentrations (0.1 to 1 ppm) of fatty acids in Precambrian rocks. Although they regard these fatty acids as being indigenous, it is possible that the sediments have been contaminated in situ by recent biogenic material. Smith et al[79] have analysed the soluble lipid components of several Precambrian and Phanerozoic cherts and on the basis of porosity, permeability and isotope $^{34}S/^{32}S$ ratios they suggest that post-depositional contamination has occurred.

The stereochemistry of some of the isoprenoid acids from Green River Shale has been determined to provide further evidence that they are derived from e.g. the phytyl group of chlorophyll or the lipids of halophilic bacteria. Eglinton et al[80] have reported the relative stereochemistries of the methyl esters of the five isoprenoid acids C_{14}, C_{15}, C_{16}, C_{19} and C_{20} isolated from Green River Shale. They showed that the C_{15}, C_{16}, C_{19} and C_{20} acids had predominantly the D

configuration at carbon positions 7 and 11. This was found to be in agreement with natural phytol which is known to have the D configuration at carbons 7 and 11. Phytanic acid, derived from phytol by reduction and oxidation, would have the D configurations at positions 7 and 11, but unless a stereospecific reduction is invoked, the product would yield the pair of enantiomers LDD/and DDD. Naturally-occurring C_{19} and C_{20} isoprenoid acids have LDD/DDD ratios of about 1-10 and 0.5 - 3.5 respectively. Values of the geological C_{19} and C_{20} acids showed LDD/DDD ratios of about 1:1 and 0.7:1.

The most important conclusions to be drawn are that these acids are of biogenic origin and that their asymmetric centres have not been racemised during their burial in the sediment. If the acids had been produced by non-stereospecific abiogenic processes, eight diastereoisomers could theoretically have been formed.

(b) MARL SLATE, AND ITS LATERAL EQUIVALENTS IN NOTTINGHAMSHIRE AND THE NORTH SEA (KUPFERSCHEIFER)

The Marl Slate samples of County Durham, and their lateral equivalents in Nottinghamshire and the North Sea area, were demineralised and the kerogens analysed for insoluble organic carbon. The percentage of insoluble organic carbon was found to be variable, ranging from 0.4% (Kneesall sample, Nottinghamshire) to 7.7% (Fishburn sample, Co. Durham) per unit weight of rock. Analyses of the eight samples are recorded in Table 2. Successive demineralisations of the shale did not totally remove all of the inorganic matrix. All of the Marl Slate samples contained substantial amounts of framboidal pyrite which, as Hirst and Dunham have remarked[9], is closely associated with the insoluble organic material. Chemical methods for removing pyrite from kerogen concentrates are known[81], but they also oxidize, or reduce, the kerogens to varying degrees. The Marl Slate samples show quite high amounts of insoluble organic carbon, ranging from 4.8%

to 7.7%, which are comparable to the Scottish oil shales[73] and the Green River Shale which contain 12.3% and 12.4% insoluble organic carbon respectively. The only exception in the samples is the Mainsforth sample (1.97% insoluble organic carbon), which was obtained from an horizon ten inches above the base of the Marl Slate. The Nottinghamshire and North Sea equivalents are more calcareous sediments and the samples analysed had less than 1% insoluble organic carbon. It is thought however that this difference does not represent varying rates of deposition of organic sapropel, but rather a difference in the precipitation of calcareous and dolomitic limestones in different areas in the basin of sedimentation.

The amount of soluble organic material extracted from each sample has been determined, and each extract has been further separated by means of column chromatography on alumina. The fractions eluted have been recorded in the experimental section. The total extracts have been recorded as percentages of the kerogen contents of the sample, since they may reflect the effect of the geothermal gradient on the stability of kerogen. The results are shown in Table 2.

Since the Marl Slate is underlain by the Permian Yellow Sands and overlain by the Magnesian Limestone, it is unlikely that migration of soluble organic material into the sequence has occurred, since both of these horizons are more permeable to ground water than the Marl Slate. Low molecular-weight organic material generated from the kerogen may have migrated outside the shale to the more porous and permeable horizons. Such a process would result in a low soluble organic/ insoluble organic ratio as is apparent in sample K-1. A high ratio would be indicative of little or no migration from the shale, and/or a process occurring, e.g. thermal heating, which would tend to produce low molecular-weight organic material from the kerogen e.g. sample K-2.

TABLE 2. INSOLUBLE ORGANIC CARBON, SATURATED HYDROCARBONS AND THEIR RELATIONSHIPS IN THE MARL SLATE AND KUPFERSCHIEFER SAMPLES

SOURCE OF SAMPLE	% Insoluble Organic Carbon	Soluble Organic Matter / Insoluble Organic Carbon	PET.ETHER ELUATE* as a % of Soluble Organic Matter
MARL SLATE (DOWNHILL Co. Durham)	6.72	0.053	14.6
DC 322 (MIDDLE STOTFOLD BOREHOLE, Co. Durham)	4.81	0.110	18.3
BU 4930 (FISHBURN NO. 1 BOREHOLE, Co. Durham)	7.68	0.071	18.6
YFF 1626 (MAINSFORTH COLLIERY No. 5 BOREHOLE, Co. Durham)	1.97	0.146	13.1
BQ 7276 (KNEESALL BOREHOLE, Notts.)	0.41	0.712	7.0
BK 9325 (MILTON BOREHOLE Notts.)	0.68	0.122	3.90
K-1 (KUPFERSCHIEFER, borehole sample, North Sea)	0.56	0.011	3.50
K-2 (KUPFERSCHIEFER, borehole sample, West Emsland)	0.81	0.489	15.0

* The saturated hydrocarbons, (petroleum ether eluates), have been tabulated as percentages of the total extractable organic material

The Marl Slate samples (Co. Durham) show rather variable soluble organic/insoluble organic ratios varying from 0.053 to 0.146 and it is doubtful if any useful conclusions about migration of lipids can be drawn from these figures. The percentages of saturated hydrocarbons contained in the total organic extracts, however, are more constant, varying from 13.1% to 18.6%. None of these samples are presently buried to a depth of more than 1,000 feet and it is unlikely that they have been affected thermally due to depth of burial or by igneous intrusions. The Nottinghamshire samples show rather low amounts of saturated hydrocarbons in their organic extracts (7.0% and 3.9%). The petroleum-ether eluates of these eight samples, after examination by i.r., t.l.c. and g.c. were shown to contain only saturated normal, branched and cyclic alkanes. High values of 13.1% to 18.6% show increasing alteration of the precursor organic compounds to more saturated and lower molecular weight hydrocarbons. Thermal effects due to greater depth of burial of the Kupferschiefer samples, K-1 and K-2, are pronounced and will be dealt with later in this discussion.

Robinson and Dinneen[82] noted that there appeared to be no consistent relationship between the ratio of soluble organic material and the total organic carbon of oil shales. The twelve oil shales that were analysed by these authors, however, were from different environments including lacustrine, marine, lagoon-marine and deltaic sediments. It is quite probable that differences were due essentially to the different types of source material.

The floral and faunal evidence from the Marl Slate and its lateral equivalents is similar, and it can reasonably be assumed that the differences in extraction ratios, Table 2, are due to the different post-depositional histories of those sediments. It is apparent from the very high extraction ratios of K-2 that the organic carbon in this horizon has been affected geothermally. The hydrogenolysis experiments, which are discussed later, will show that kerogen may be easily

degraded to petroleum-like constituents at geologically feasible temperatures ($\sim 175^{o}C$).

INFRA-RED SPECTROPHOMETRY:

Petroleum-ether eluates: all the samples showed typical alkane absorption with bands at 2970 (strong, ν_{as} CH_3), 2920-2925 (strong, $\nu_{as}CH_2$), 1464-1465 (δ CH_2, δ_{as} CH_3), 1376 (medium δ_s CH_3) and 720 ($-(CH_2)_n$ rock) cm^{-1}. No carbonyl or olefinic bands were present. The infra-red spectrum of sample K-2 changed slightly during consecutive runs due to evaporation of low-boiling components ($< C_{13}$), which were present in unusually high concentration in this sample.

Benzene eluates: all the samples were very similar except for sample K-1, which yielded a negligible benzene eluate. Absorption bands at 3115 (=CH-), 1613 and strong broad bands at 810 and 747 cm^{-1} suggested substituted aryl groups and alkyl substituents were suggested by the bands at 2970, 2920, 1465 and a shoulder at 725 cm^{-1}. These i.r. data suggest the presence of a mixture of alkyl benzenes. G.C. analysis of these fractions with high efficiency columns showed them to be extremely complex with no prominent components.

Chloroform eluates: all the samples were qualitatively similar, except for the Fishburn sample, which showed slight hydroxyl absorption at 3370 cm^{-1} and absorption at 1210 cm^{-1} suggesting a phenol. Aromatic bands at 3012, 1610 and 750 cm^{-1} were present in these samples. Several weak but sharp bands occur between 990 cm^{-1} and 1210 cm^{-1}, which with the strong bands noted above suggest that polysubstituted aromatic nucleii are present. Porphyrins are present in these eluates, but not in sufficient amounts to cause significant absorption above the large background absorption in the 2300-2500 cm^{-1} region. Strong absorption at 2980, 2920, 1460 and 1375 cm^{-1} showed that aliphatic chains were still present in the later eluates.

Methanol eluates: all the samples showed once again aliphatic bands at 2980, 2925, 1460, 1375 cm^{-1}, and aromatic bands at 3010,

1510-1640 and 750 cm^{-1}, which were reduced in intensity. Two broad bands of medium intensity at 1092 and 1020 cm^{-1} were present which were difficult to characterise.

The i.r. analyses of these samples show several important features.

1) The presence of aliphatic structures is evident in all of the samples and their sub-fractions.

2) There are no aromatic components in the petroleum ether eluates (alkyl benzenes may be eluted from alumina columns with petroleum ether), but considerable amounts are present in the remaining eluates.

3) There are no significant amounts of carboxyl and carbonyl containing compounds in the eluates.

Various workers (see Chapter VIII) have ascribed the loss of carboxyl groups from diterpene and triterpene acids in fossil woods as being due to prolonged burial and Robinson and Dinneen[82] have shown that Tertiary Oil Shales are richer in carboxyl and hydroxyl groups than older oil shales.

I.r. analyses of the kerogen concentrates were not reliable, since it was difficult to remove all the silicate and other inorganic minerals. The Downhill kerogen concentrate had an organic carbon content of 42.0% and this was the only sample from which an i.r. spectrum was obtained. The spectrum gave bands below 1200 cm^{-1} which were of little significance due to absorption by the inorganic matrix. The presence of silicates was shown by the presence of bands at 1100, 1017, 1005, 908 and 694 cm^{-1} [73]. Broad bands occurred at 2950, 1450, and 1375 cm^{-1} indicating the presence of aliphatic groups and alkyl substituents and a broad band at 1625 cm^{-1} suggested that unsaturated material was present. Weak absorption at 3400 cm^{-1} showed a small contribution due to hydroxyl groups and an absorption

band at 1700 cm^{-1} showed the presence of carboxyl groups or ester groups in the kerogen. Robinson and Dinneen[143] have stressed, however, that absorption in this region does not appear to be consistent with values obtained chemically for carboxyl and ester oxygen from the oil shales they analysed.

HYDROCARBONS IN MARL SLATE AND ITS LATERAL EQUIVALENTS

Separation of the total alkane fractions, obtained from each of the eight samples, into normal and branched-cyclic fractions, was not possible with all of the samples due to lack of material. Since each of the Nottinghamshire samples yielded less than 4 mg. of alkanes, it was decided to analyse them by g.c. only. The Kupferschiefer samples K-1 contained only 0.6 mg. of alkanes after sulphur had been removed[83] on a column of spongy copper. Furthermore the Kupferschiefer sample K-2, although very rich in alkanes ($>$ 600 p.p.m.), was unusual in that its constituent normal alkanes reached a maximum concentration below nC_{13}; 5A molecular sieving of this fraction would have caused a loss of more than 80% and therefore it was not attempted.

All of the samples with the exceptions noted, were extracted and chromatographed in the same manner. The Kupferschiefer sample K-2 was extracted with pentane ultrasonically and chromatographed as before. This procedure allowed recovery, and analysis by gas liquid chromatography, of hydrocarbons below nC_{10}.

The procedure, described in this thesis, for isolating hydrocarbons allows their extraction below nC_{15}. Evaporation of solvents under reduced pressure (20 mm Hg) at 40° - 50°C caused substantial losses of alkanes up to nC_{15}. Therefore, the figures recorded for the total alkanes of these samples represent minimum values due to the above noted limitation of the procedure. However, since the same procedure was adopted throughout, qualitative differences can be shown between the samples as discussed previously. In the case of the Kupferschiefer sample K-2, where the difference in distribution

is towards the low molecular-weight region, it is difficult to draw useful conclusions about the concentration of alkanes. The concentrations of total alkanes in the samples are recorded below in Table 3 (cf. Table 2 for description of samples).

TABLE 3. TOTAL ALKANE CONTENT OF THE MARL SLATE AND ITS LATERAL EQUIVALENTS

SAMPLES	% INSOLUBLE ORGANIC CARBON	TOTAL ALKANES (p.p.m.)
Marl Slate Downhill	6.72	520
DC 322	4.81	970
BU 4930	7.68	1020
YFF 1626	1.97	380
BQ 7276	0.41	32
BK 9825	0.68	72
K-1	0.56	6
K-2	0.81	590

It is evident that there is no correspondence between the amount of total alkane extract of a sample and its insoluble organic carbon content. However, when the total alkane,normal, and branched-cyclic alkane fractions were examined by g.c., it was apparent that the Marl Slate samples (and their Nottinghamshire equivalents) were qualitatively similar. The gas chromatograms (Figs 7-10) for the Marl Slate (Downhill), DC 322 323, BU 4930 and YFF 1626 samples show marked similarities. They all contain the isoprenoid hydrocarbons, phytane and pristane together with the lower C_{14}, C_{15}, C_{16} and C_{18} homologues. The triterpane or sterane peaks in each sample, although unidentified, have identical retention indices and are probably the same hydrocarbons. It is also evident from the g.c. of the total alkanes extracted from the Magnesian Limestone (Fig. 11) that the distribution of hydro-

Fig. 7

Saturated hydrocarbons from the Marl Slate (Downhill)

A. Normal alkanes

Chromatographic conditions: column 20 feet x 0.040", packed with 3% OV-1 on Gas Chrom. Q(100/120 mesh). Oven programmed at 4°C/min. between 100°-300°C. Nitrogen carrier gas, 8 ml/min. Varian Aerograph 1200. Detector temperature 310°C, injector temperature 300°C.

B. Total alkanes

Chromatographic conditions: as above.

C. Branched-cyclic alkanes

Chromatographic conditions: as above.

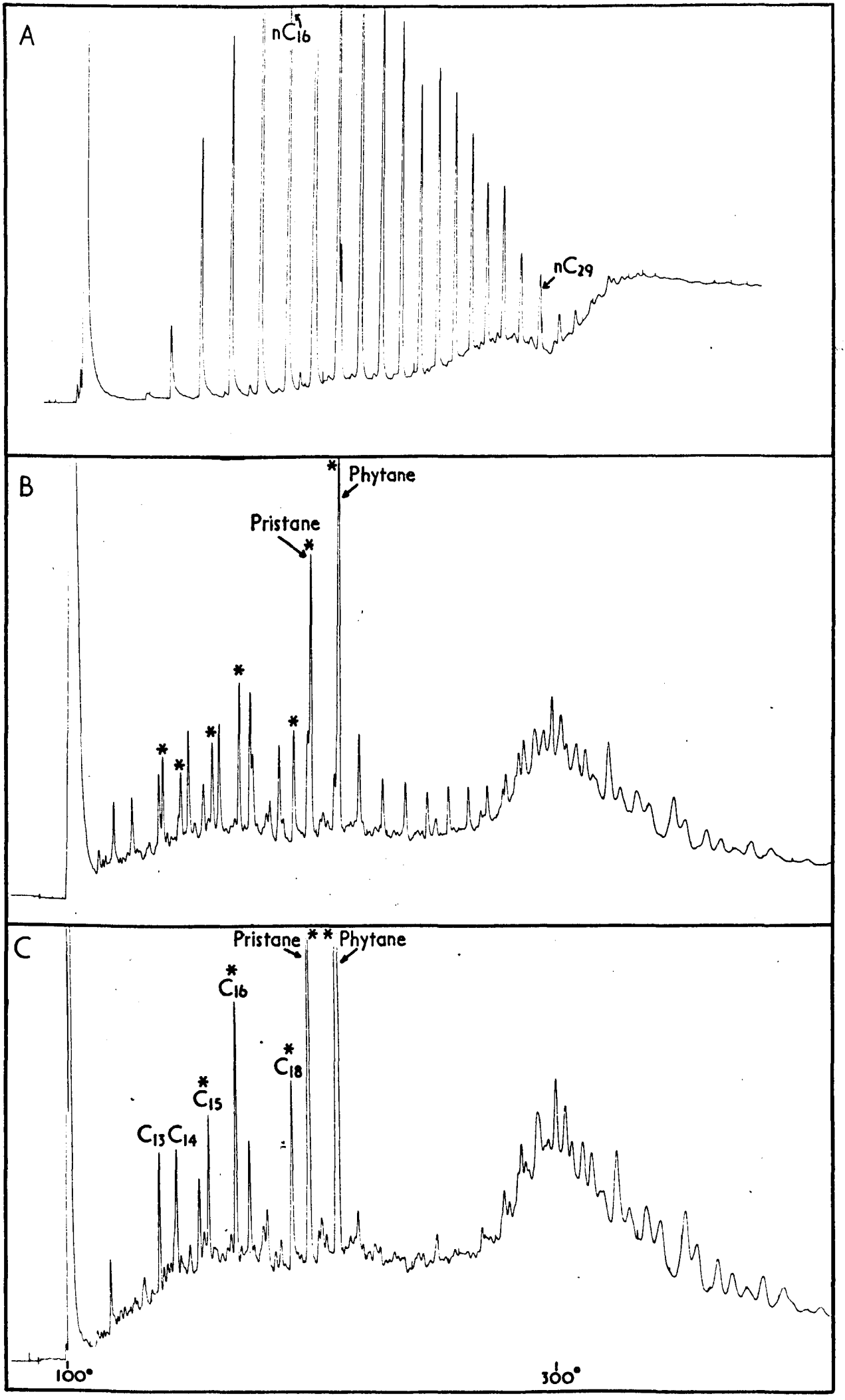

A
nC16
nC29
B
Phytane
Pristane
C
Pristane
Phytane
C16
C18
C15
C13 C14
100°
300°

Fig. 8

Saturated hydrocarbons from the Middle Stotfold borehole (DC322)

A. Total alkanes

Chromatographic conditions: see Fig. 7.

B. Branched-cyclic alkanes

Chromatographic conditions: as above.

C. Normal alkanes

Chromatographic conditions: as above.

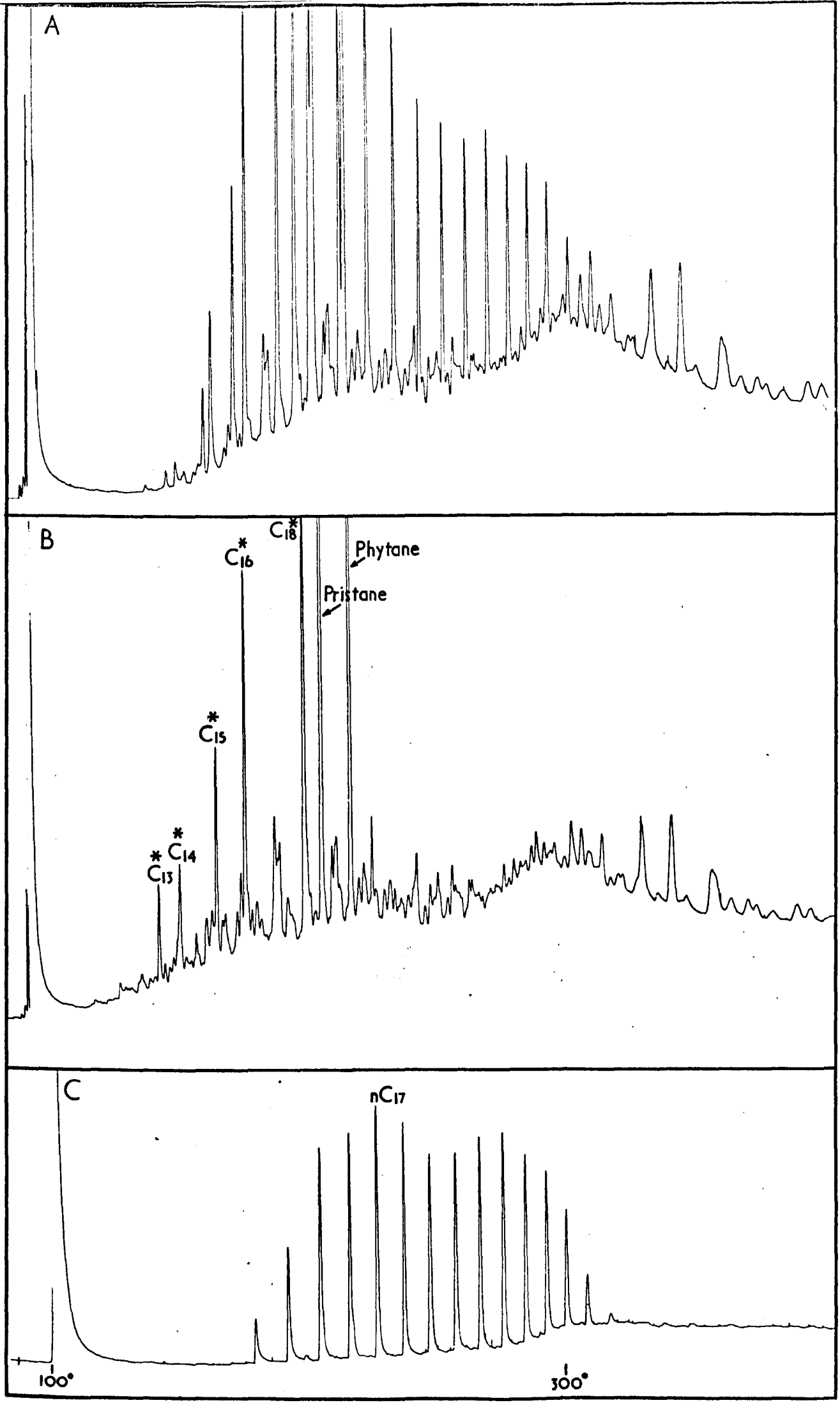

A
B
C18*
C16*
Phytane
Pristane
C15*
C14*
C13*
C
nC17
100°
300°

Fig. 9

Saturated hydrocarbons from the Fishburn borehole (BU 4930)

A. Total alkanes

Chromatographic conditions: see Fig. 7.

B. Branched-cyclic alkanes

Chromatographic conditions: as above.

C. Normal alkanes

Chromatographic conditions: as above.

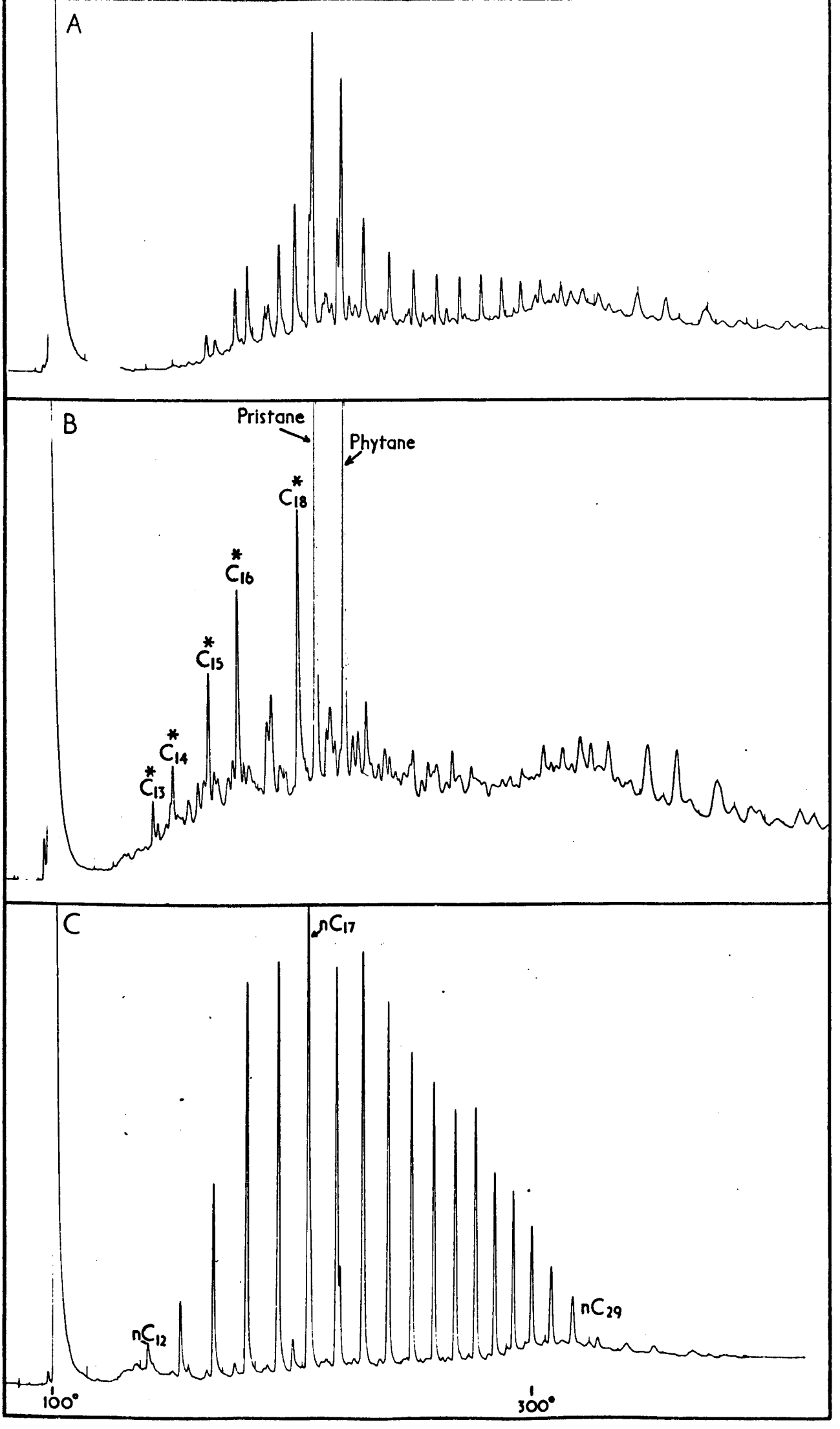

A
B
Pristane
Phytane
*C18
*C16
*C15
*C14
*C13
C
nC17
nC12
nC29
100°
300°

Fig. 10

Saturated hydrocarbons from the Mainsforth Colliery borehole (yFF 1626)

Total alkanes

Chromatographic conditions: see Fig. 7.

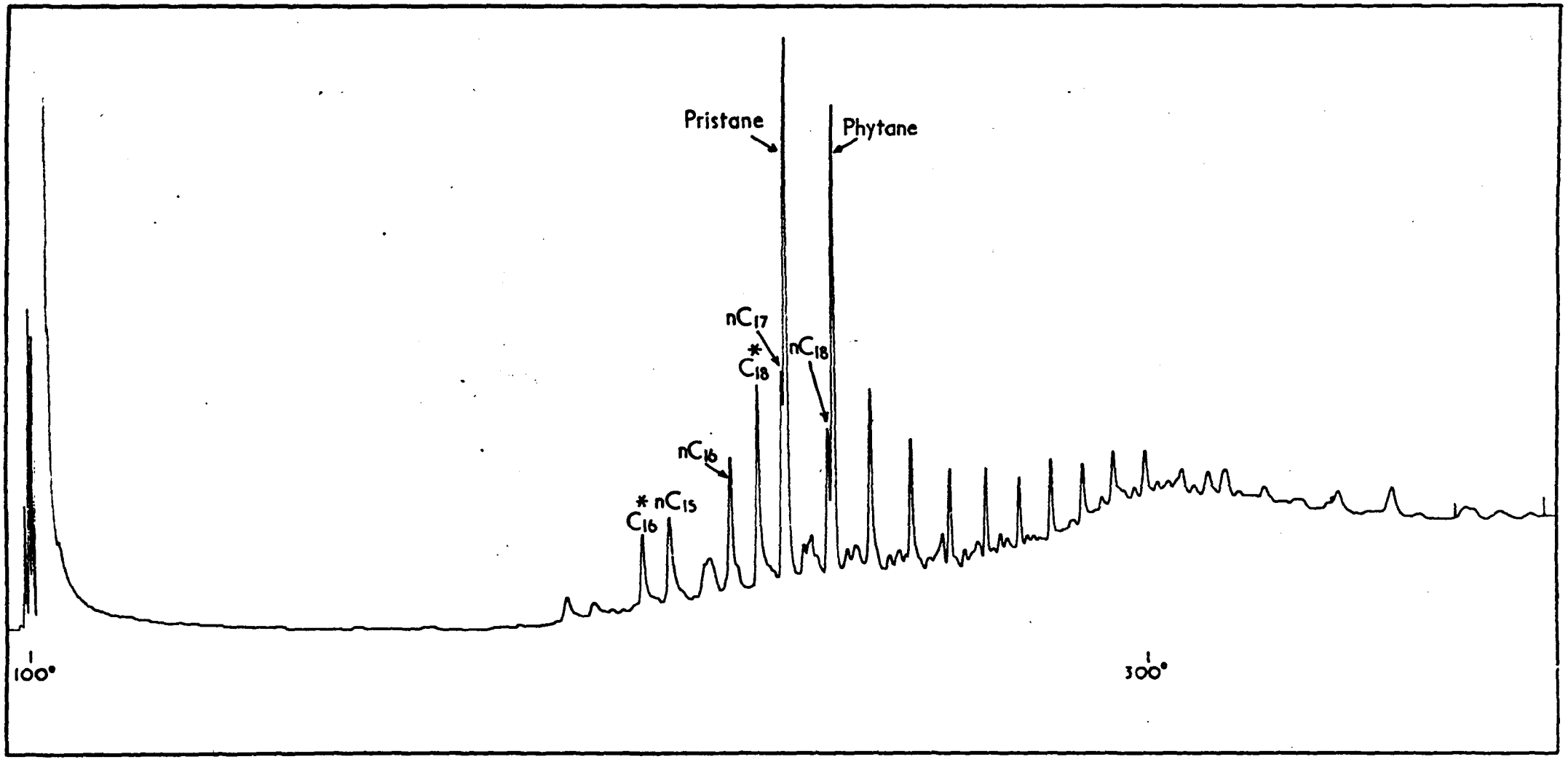
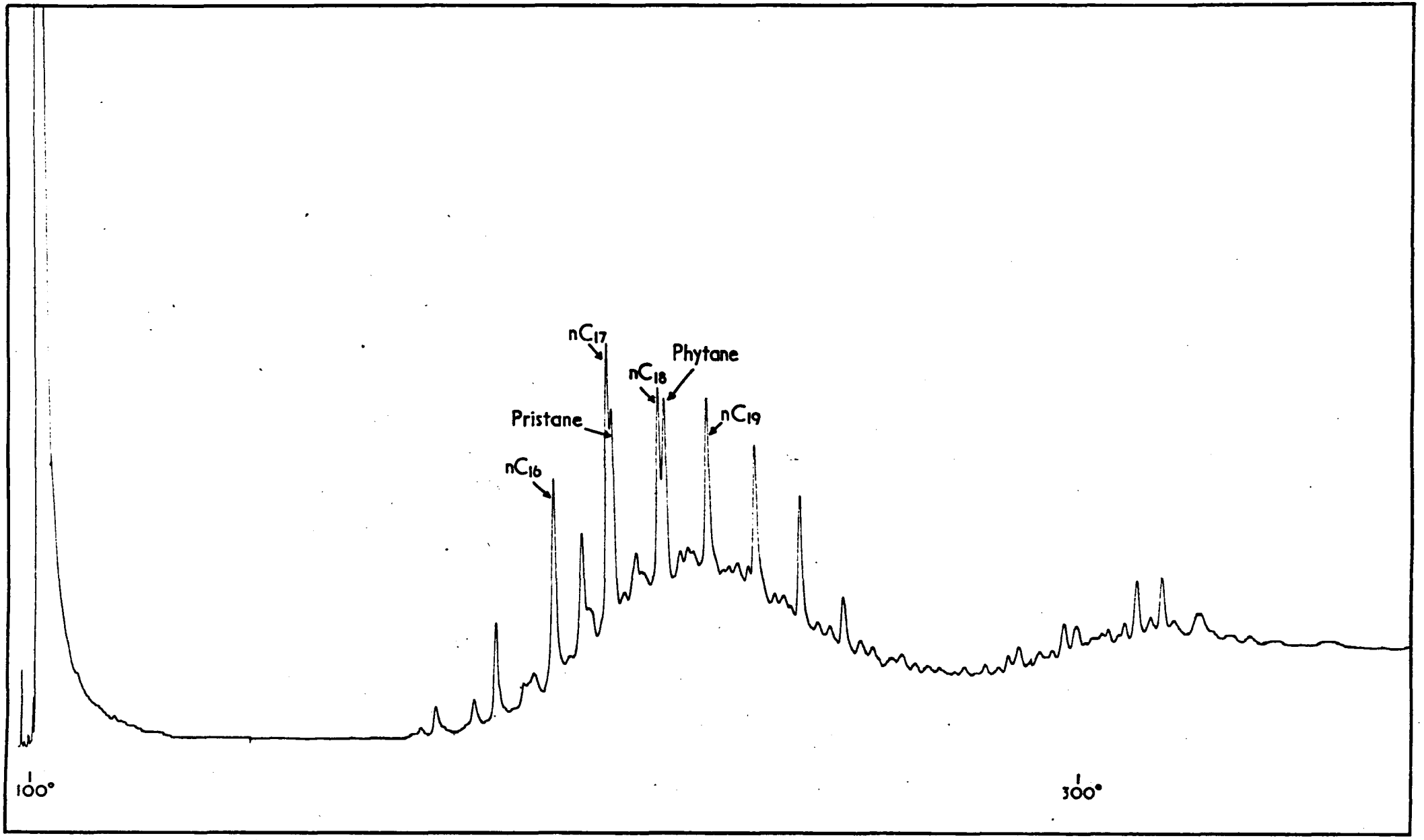

Pristane
Phytane
nC17
*
C18
nC18
nC16
* nC15
C16
100°
300°

Fig. 11

Saturated hydrocarbons from the Magnesian Limestone (Downhill)

Chromatographic conditions: see Fig. 7.


nC17
Phytane
nC18
Pristane
nC19
nC16
100°
300°

carbons is also similar to those of the Marl Slate samples. The concentration of total alkanes in the limestone is however less than 10 p.p.m.

The acyclic hydrocarbons were collected individually by preparative g.c. and the mass spectra of the five Downhill isoprenoids are shown in Fig. 12. The spectra shown have established the molecular weights of the isoprenoid hydrocarbons and also have indicated where the branches occurred.

Branched hydrocarbons fragment preferentially at the branches, as shown in Fig. 13, giving energetically favourable tertiary or secondary carbonium ions. Thus the masses from the fragmentation pattern shown in Fig. 13 can be related to the prominent fragment ions marked in Fig. 12. This can be seen in the spectrum of the C_{15} isoprenoid. The fragment ions in the m.s. of this compound can be easily calculated from the fragmentation pattern shown in Fig. 13. Pristane, the C_{19} isoprenoid, is symmetrical and can give an ion at m/e 183 in two ways. The other isoprenoids can be cleaved in only one position to give an ion at m/e 183, and so this particular ion is not as intense as in the C_{19} isoprenoid. From the fragmentation patterns of the isoprenoid hydrocarbons shown in Fig. 13, it is possible to compare the fragmentations expected[84,85,86,87] to those obtained in the spectra. These spectra compare very favourably with those of previous workers[88] for the isoprenoid hydrocarbons isolated from Green River Shale, and of authentic pristane and phytane.

It has been shown however that the mass spectra of some branched-chain hydrocarbons can be ambiguous. McCarthy et al.[57] have synthesised acyclic hydrocarbons containing methyl branches differing from those in phytane (2,6,10,14-tetramethylhexadecane) in the position of only one methyl substituent e.g. 3,6,10,14-tetramethylhexadecane.

Figure 12

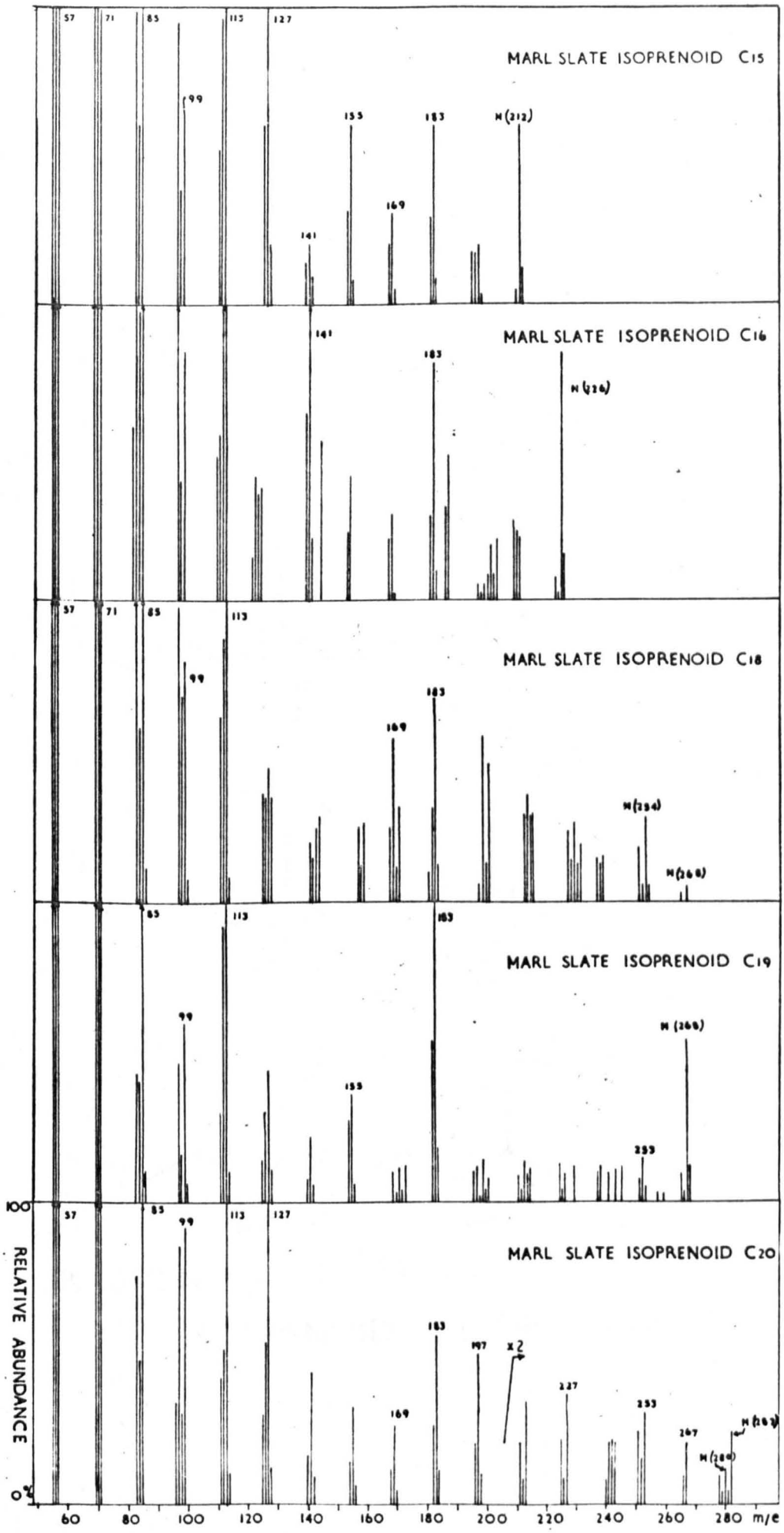
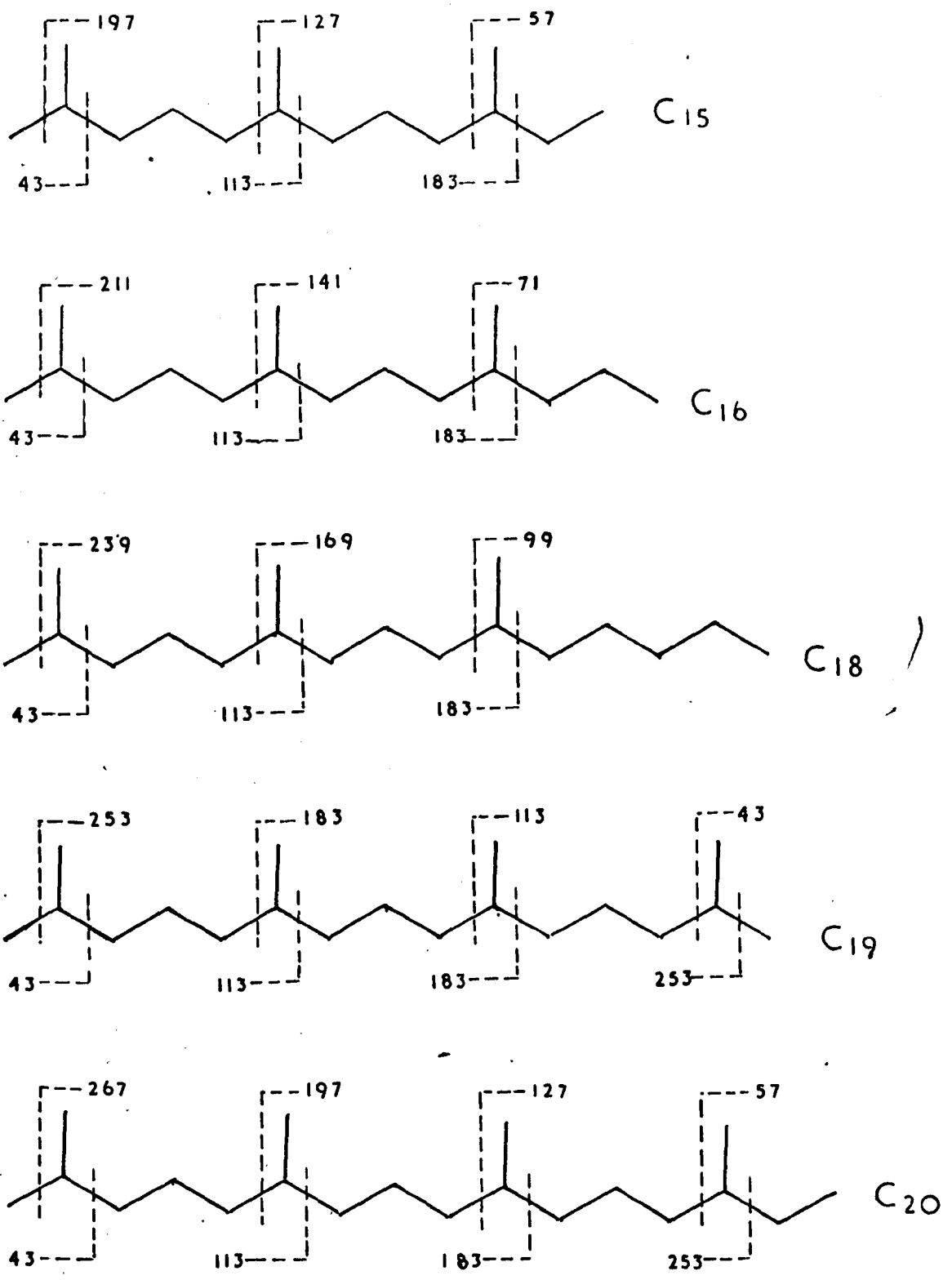
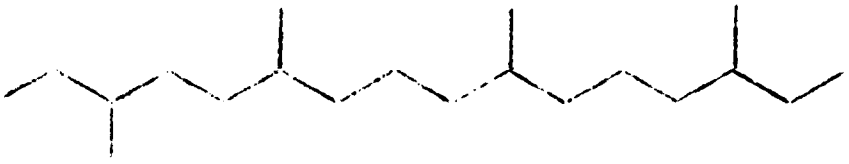

MARL SLATE ISOPRENOID C15
MARL SLATE ISOPRENOID C16
MARL SLATE ISOPRENOID C18
MARL SLATE ISOPRENOID C19
MARL SLATE ISOPRENOID C20
RELATIVE ABUNDANCE
m/e

Figure 13



FRAGMENTATION PATTERN OF THE C_{15}—C_{20} ISOPRENOID HYDROCARBONS



3,6,10,14 - tetramethylhexadecane

Such hydrocarbons show almost identical mass spectra to the regular isoprenoids and because of this McCarthy et al have suggested that the most convenient way of differentiating the two is by their slightly different g.c. retention indices measured on high efficiency capillary columns. Thus mass spectral identity and identical retention indices on low resolution columns, do not necessarily confirm the presence of these acyclic isoprenoids. The retention indices of the Marl Slate (Downhill) acyclic isoprenoids were calculated from programmed analyses on 20ft x 1/16" O.D. high efficiency packed columns coated with 3% OV-1 on Gas Chrom Q, generating 10,000-13,000 theoretical plates. These compare very well with the retention indices of the Green River Shale isoprenoid hydrocarbons on the same columns and it is reasonably certain that the acyclic isoprenoids in the Marl Slate are similar to those in the Green River Shale. The retention indices of the Marl Slate and Green River Shale acyclic hydrocarbons, determined on the 20ft columns, are shown in Table 4.

The Nottinghamshire samples contain small amounts of alkanes compared to the Co. Durham samples but the gas chromatograms show that they have a similar distribution of the acyclic isoprenoid hydrocarbons, (Fig. 14). The normal alkanes in each of the Nottinghamshire samples range from $\underline{n}C_{14}$ to $\underline{n}C_{24}$, as is seen in the

Fig. 14

Saturated hydrocarbons from the Kneesall borehole (BQ 7276) and the Milton borehole (BK 9825)

A. Total alkanes (BQ 7276)

Chromatographic conditions: see Fig. 7.

B. Total alkanes (BK 9825)

Chromatographic conditions: as above.

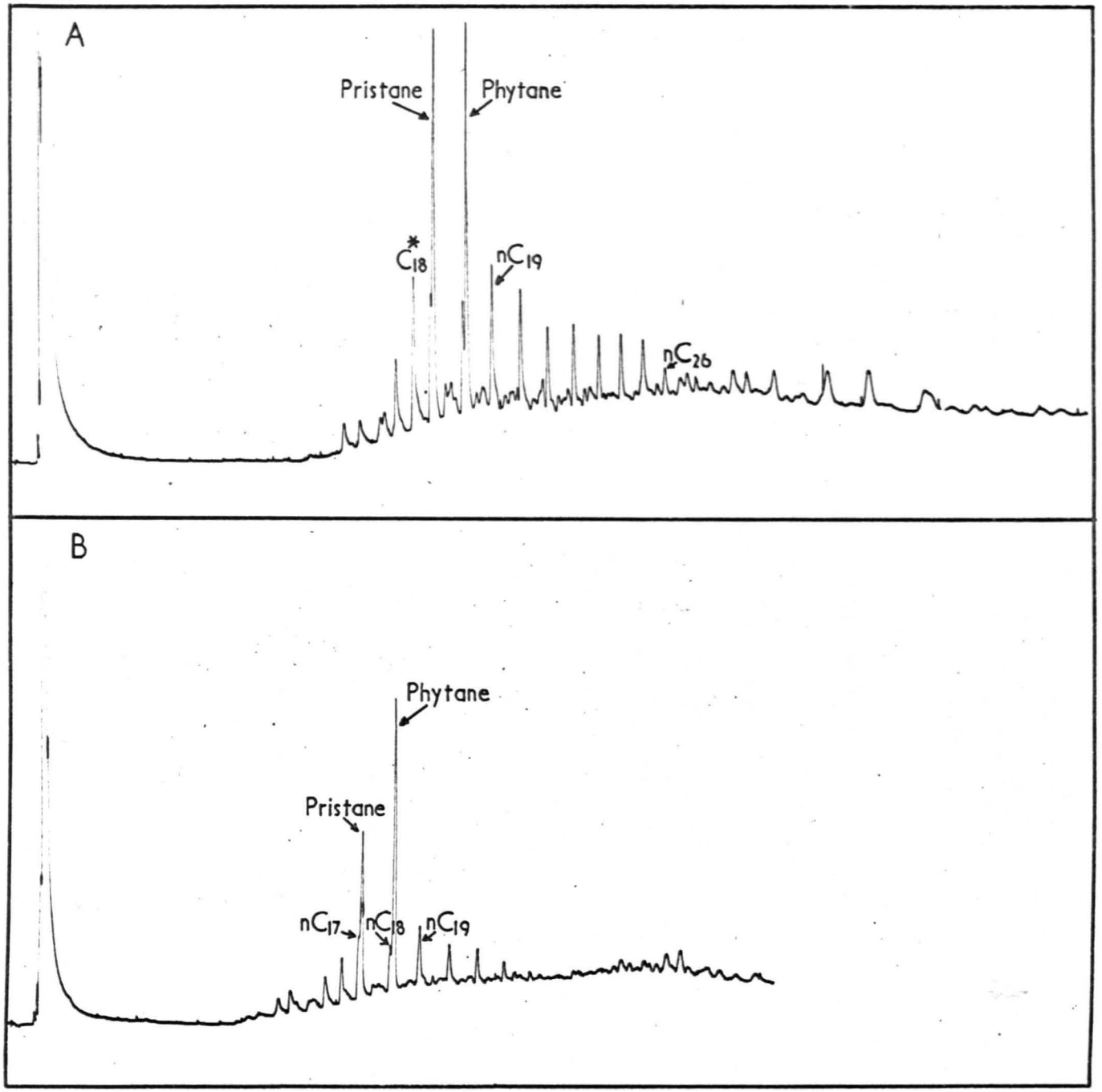

A
Pristane
Phytane
C*18
nC19
nC26
B
Phytane
Pristane
nC17
nC18
nC19

TABLE 4. KOVATS RETENTION INDICES OF ISOPRENOID HYDROCARBONS IN THE GREEN RIVER SHALE AND THE MARL SLATE (ON OV-1)

CARBON NUMBER	GREEN RIVER SHALE	MARL SLATE (DOWNHILL)	AUTHENTIC* STANDARDS
C_{13}	1215	1215	
C_{14}	1276	1275	
C_{15}	1378	1379	1376
C_{16}	1466	1465	1464
C_{18}	1653	1652	1651
C_{19}	1709	1709	1709
C_{20}	1814	1816	1819
C_{21}	1916	1914	

*Data provided by Mr. K. Hall, University of Newcastle

total alkane chromatograms of each sample, with distribution maxima at $\underline{n}C_{17}$ and $\underline{n}C_{19}$ respectively.

The Marl Slate samples show a normal alkane distribution varying from $\underline{n}C_{13}$ to about $\underline{n}C_{30}$ with a maximum concentration at $\underline{n}C_{17}$ or $\underline{n}C_{19}$ suggesting a derivation from marine and/or lower organisms. Only minor amounts of the $\underline{n}C_{27}$ and $\underline{n}C_{29}$ alkanes are present suggesting little contribution from terrestrial plants. Although there is microscopical evidence for the presence of spores in these samples they are only minor contributors to the total organic material present. In the DC 322 323 sample, Fig. 8, a bimodal distribution of normal alkanes is apparent with envelope maxima at $\underline{n}C_{17}$ and $\underline{n}C_{22}$, the normal alkanes at $\underline{n}C_{27}$ or above occur only as minor contributors. The effect in the gas chromatograms is exaggerated to a certain degree by the amount of column bleed present. In all of these samples a smooth envelope of normal alkanes is present having a CPI value close to unity (1.04 for the Downhill sample).

Significant changes in the distribution of total alkanes are evident when one follows the lateral equivalents of the Marl Slate eastwards under the North Sea where the sediments have been buried to more than 8,000 feet. Thus, a change in distribution of normal alkanes is evident in the Kupferschiefer K-2 sample which has an envelope maximum below $\underline{n}C_{13}$ (Fig. 16). No evidence of pristane, phytane or triterpanes is present in this chromatogram. The Marl Slate equivalents, represented here by the Kupferschiefer samples K-1 and K-2, have been affected by geothermal temperatures of around 100°C for prolonged periods, assuming a geothermal gradient of 1°C per 100 ft. The alkanes present in these shales, therefore, show the effect of geothermal maturation on the same type of precursor organic material that contributed to the Marl Slate of Durham and Nottinghamshire.

The K-1 sample contained only 6.0 p.p.m. of total alkanes. The gas chromatogram of this fraction is shown in Fig. 15 and shows a complex hump of unresolved hydrocarbons on which are superposed a series of normal alkanes ranging from $\underline{n}C_{15}$ to $\underline{n}C_{24}$ with a maximum envelope at possibly $\underline{n}C_{17}$ or $\underline{n}C_{19}$. Pristane and phytane are present but they are no longer the dominant peaks that are evident in the earlier chromatograms. The K-2 sample is strikingly different and although containing twice as much organic carbon as K-1, the amount of total alkanes present is about a hundredfold greater. The chromatograms shown here, Fig. 16, represent the total alkanes recovered by (A) the normal solvent extraction procedure, and (B) by a cold pentane extraction. It is apparent that the two chromatograms are very different. A distribution of normal alkanes is present from below $\underline{n}C_{10}$ to $\underline{n}C_{30}$ but the maximum concentration of alkanes is below $\underline{n}C_{13}$. Increasing amounts of branched hydrocarbons are present below $\underline{n}C_{13}$ and it appears likely that some form of "cracking" of the longer chain hydrocarbons has occurred. No evidence of significant amounts of pristane or phytane is present and this is in agreement with the

Fig. 15

Saturated hydrocarbons in the Kupferschiefer (K-1)

Total alkanes

Chromatographic conditions: see Fig. 7.

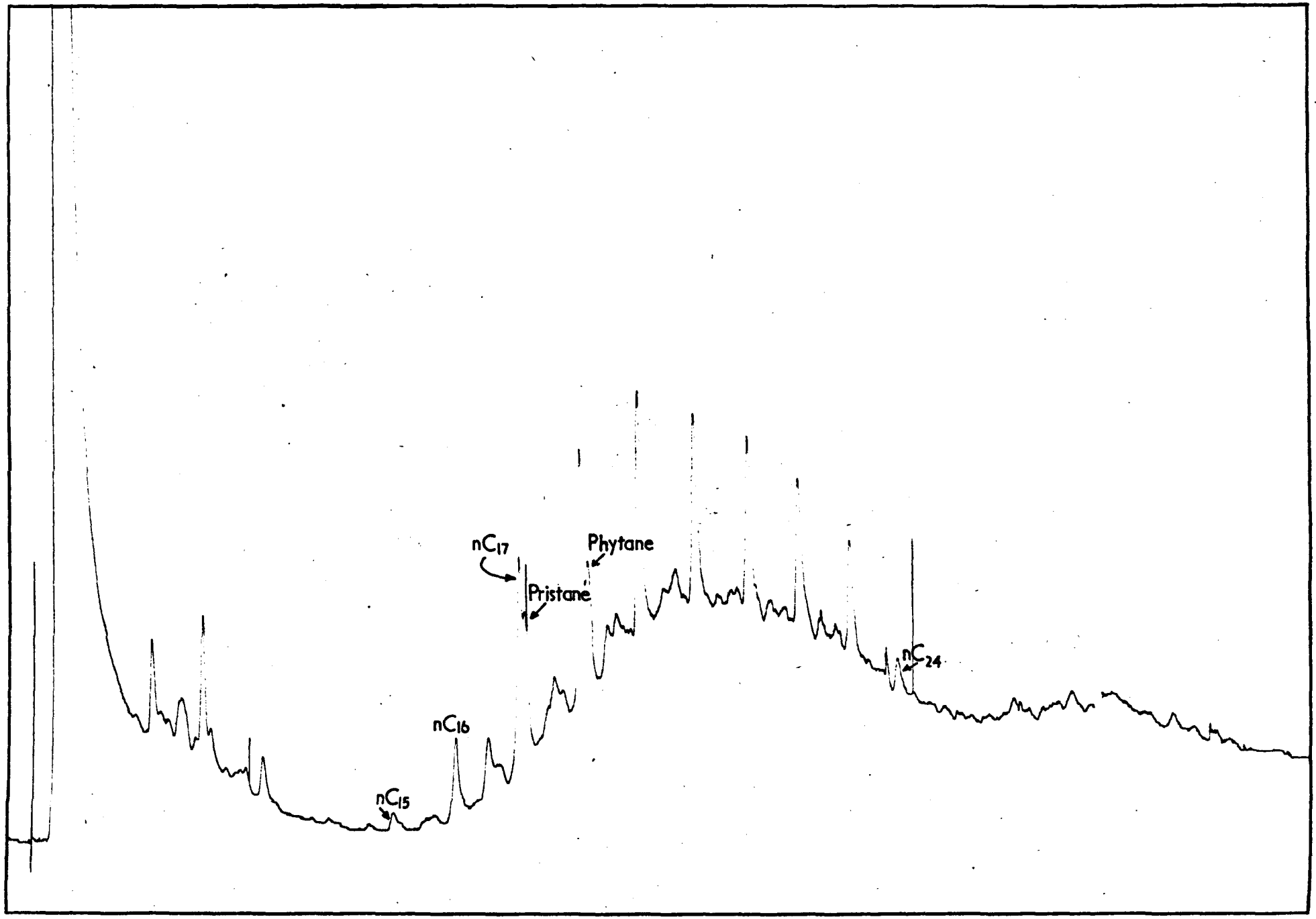

nC17
Pristane
Phytane
nC24
nC16
nC15

Fig. 16

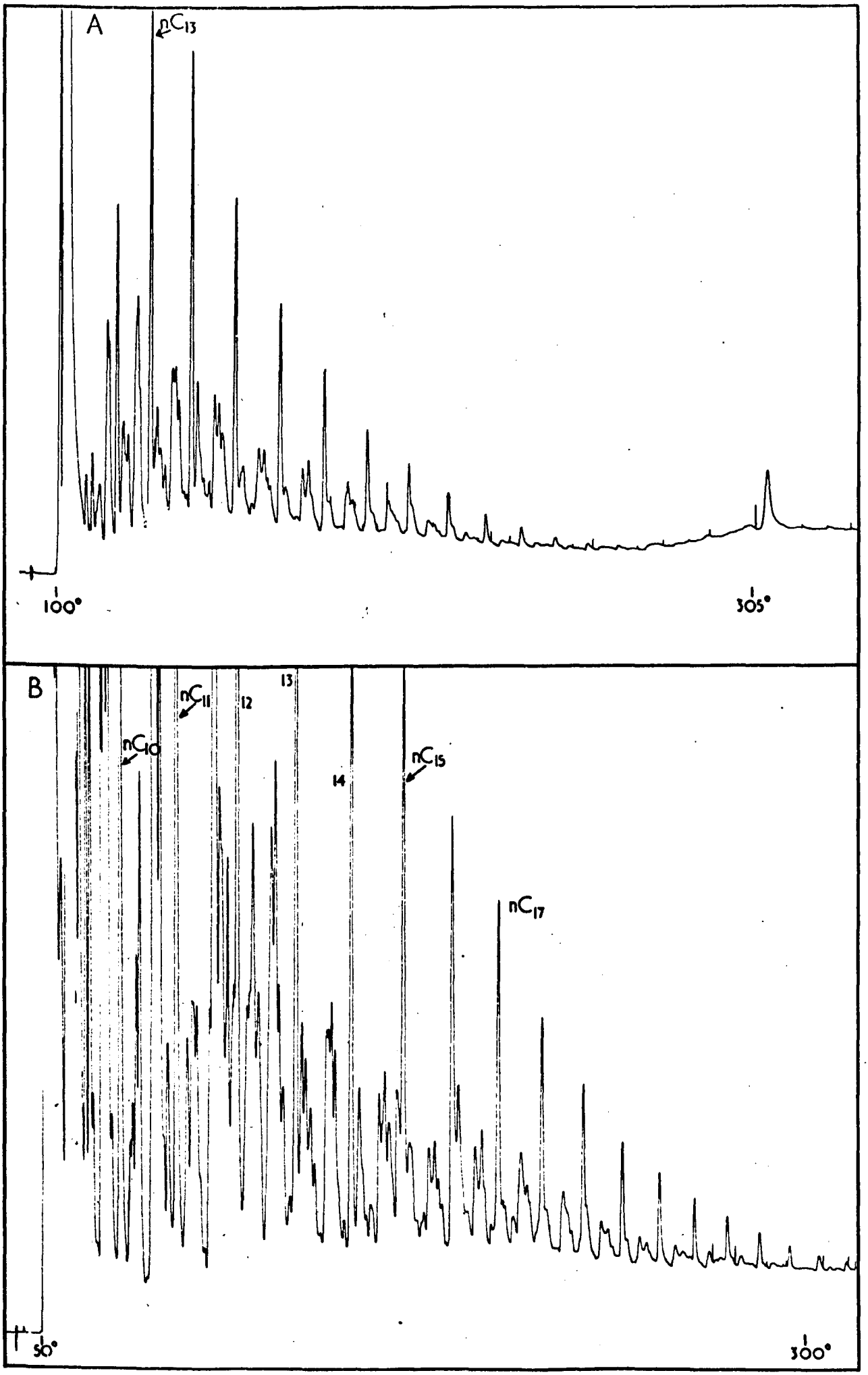
Saturated hydrocarbons in the Kupferschiefer (K-2)

A. Total alkanes (normal solvent extraction procedure)

Chromatographic conditions: as for Fig. 7 except that the oven was programmed from 100° - 305° C.

B. Total alkanes (cold pentane extraction)

Chromatographic conditions: as for Fig. 7 except that the oven was programmed from 50° - 300° C.


A
nC13
100°
305°
B
nC10
nC11
12
13
14
nC15
nC17
50°
300°

fact that thermal cracking would be energetically preferred at positions of branching.

The normal alkanes originally present in the shale could have been degraded to lower molecular weight compounds by possibly two methods (i) a radical mechanism or (ii) a carbonium ion mechanism.

(i) Radical Mechanism[34]

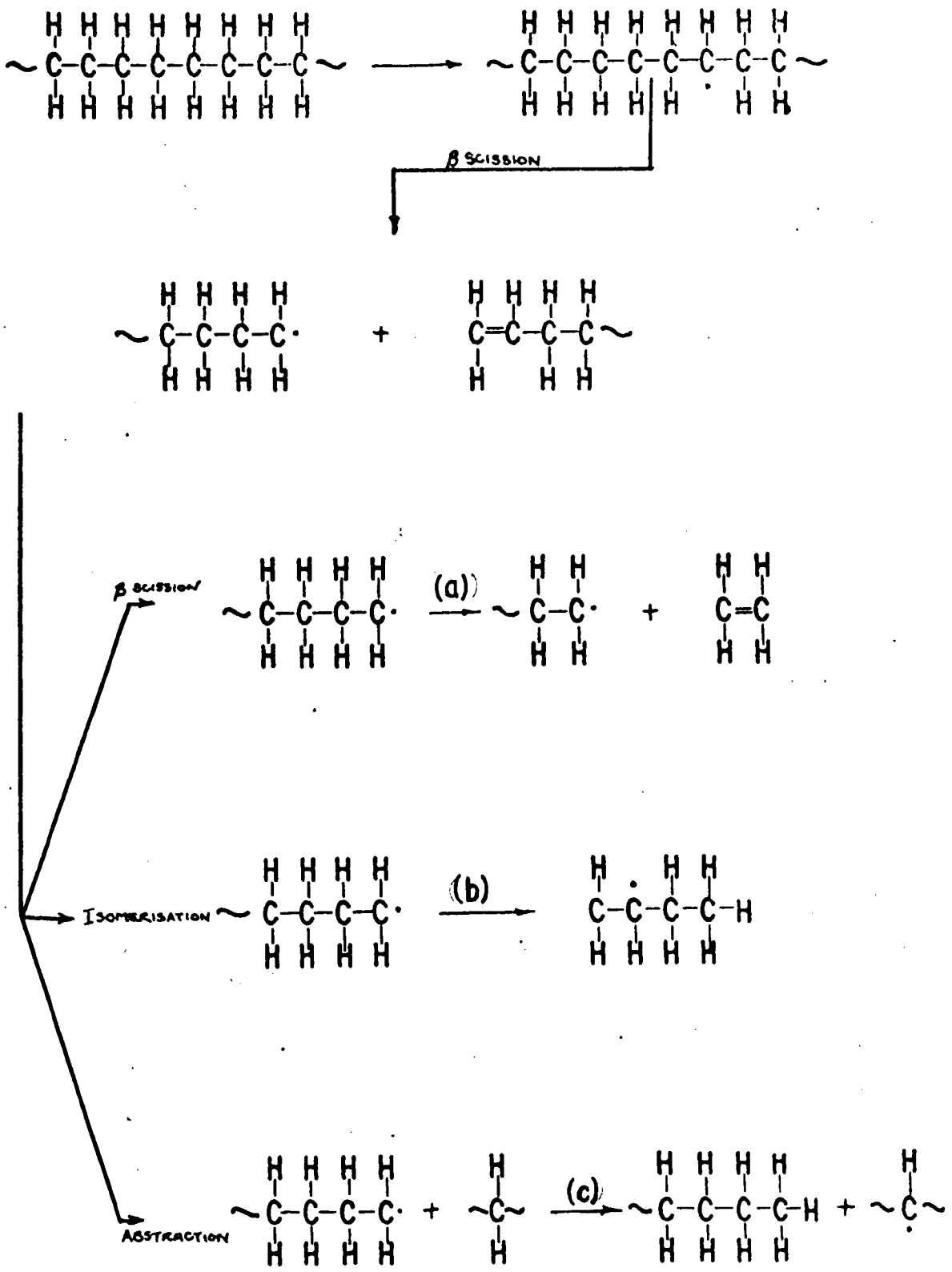
A hydrogen atom could be lost from a n-paraffin by collision (being more likely as the temperature increases). The resulting radical may undergo isomerisation to an energetically more favoured radical or it may "crack" at the carbon-carbon bond located in the β - position of the carbon lacking the hydrogen atom, giving rise to a primary radical and terminal olefin Fig. 17. The primary radical can then (a) react to give ethylene and a new primary radical or (b) isomerise or (c) abstract hydrogen from a neutral molecule resulting in a new n-paraffin and a new radical. The major reaction products are n-paraffins, terminal olefins and ethylene.

(ii) Carbonium ion mechanism

In this type of reaction catalysts such as clays and silica-alumina mixtures are essential participants. These catalysts are of the acidic type providing protons that enable the reaction to proceed.

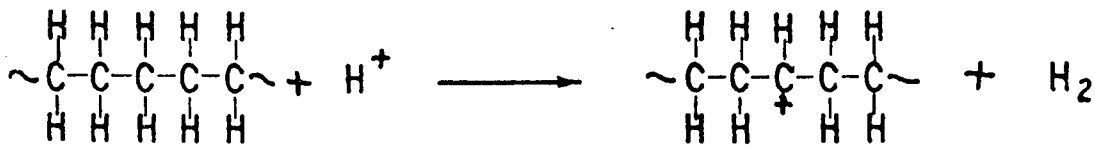
A normal paraffin may react upon collision with a proton to yield a carbonium ion which can then undergo several important types of rearrangement, and reactions, as shown in Fig. 18 (a-d). By these mechanisms it is possible to generate hydrogen within the sediment which could saturate the terminal olefins produced without having to invoke disproportionation reactions. In this type of mechanism the most favoured reaction is one in which a primary ion is cracked to yield a tertiary ion. The following data, Table 5, show the heats of cracking required to produce various carbonium ions R^+ and illustrate why it is unlikely that methyl or ethyl ions are obtained as fragments

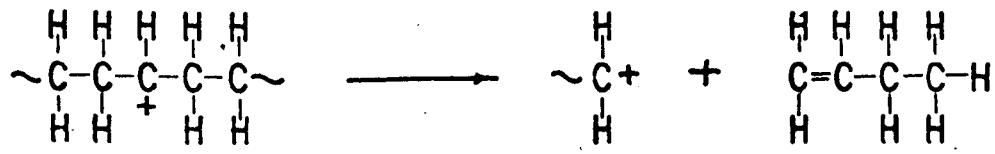
Figure 17



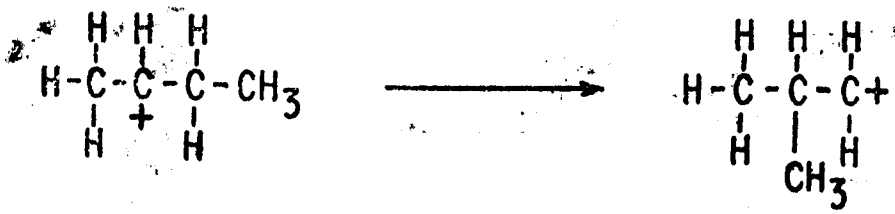
DEGRADATION OF N—ALKANES BY A RADICAL MECHANISM

Figure 18

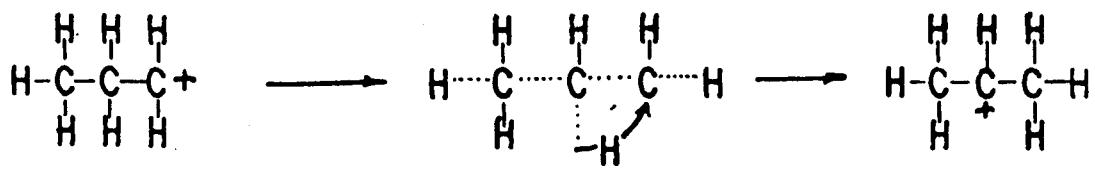




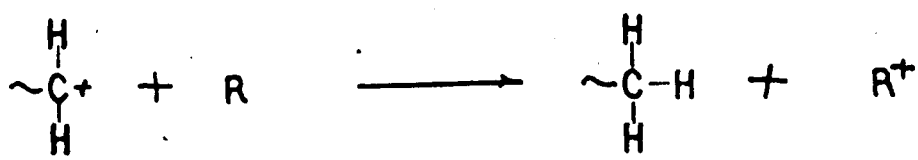
(a)



(b)



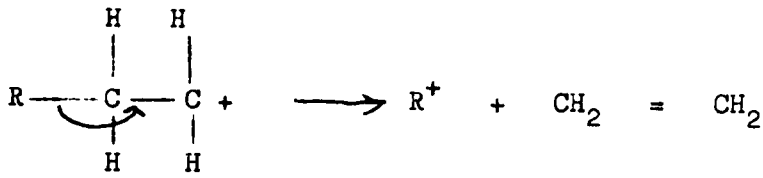
(c)



(d)

DEGRADATION OF N-ALKANES BY A CARBONIUM-ION MECHANISM

in catalytic cracking.

A 

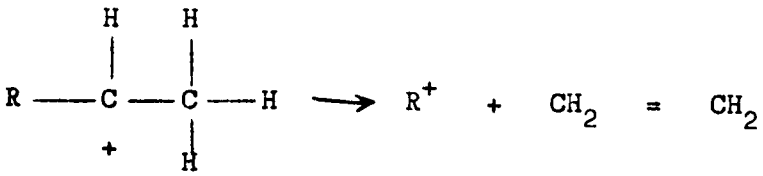
B 

TABLE 5. HEATS OF "CRACKING" OF PRIMARY, SECONDARY AND TERTIARY CARBONIUM IONS[90]

Ion R+	Reaction A ΔH_{298} Kcals/mol.	Reaction B
CH_3+	69.5	85.5
C_2H_5+	35.0	61.0
nC_3H_7+	22.5	47.5
sec. C_3H_7+	8.5	33.5
tert C_4H_9+	-7.5	17.5

The radical type of mechanism shows little skeletal isomerisation whereas branched alkanes are produced in the carbonium ion mechanism. Previous work has shown experimentally that the thermal cracking (radical mechanism) of n-octacosane generates predominantly lower molecular weight n-alkanes and terminal olefins[62]. The presence of large amounts of branched hydrocarbons in the K-2 sample suggests that long chain normal hydrocarbons have been degraded by a carbonium ion mechanism.

Jurg et al[34,90] showed that in the decarboxylation of behenic acid ($C_{22:0}$) at temperatures up to 275°C the type of low molecular weight hydrocarbons obtained suggested that carbonium ions were the intermediates in the formation of these hydrocarbons. This suggestion was based on the prominence of skeletal isomers occurring in the

reaction products. Above this temperature the authors suggested that a radical reaction became prominent. This was based on the fact that C_2 hydrocarbons, the percentage of n-alkanes and the amount of unsaturated hydrocarbons increased considerably coupled with an increase of the activation energy of the reaction. In the geological environment geothermal effects are more likely to occur below 200°C, otherwise low grade metamorphism would have occurred and considerably altered the sediments. This would favour a carbonium mechanism for unaltered sediments. Sediments which show visible signs of metamorphism, determined from their mineral assemblage, have generally been heated above ca 250°C so that the organic carbon has been reduced to graphite and the soluble organic material has disappeared.

The results of comparing the total alkanes in the Marl Slate and its lateral equivalents have established the following:

(a) Although quantitative differences occur in the organic material extracted from the Durham and Nottinghamshire samples it is apparent, from their gas chromatograms, that they are qualitatively similar suggesting that the ecological conditions during deposition were comparable.

(b) Eastwards, increased depth of burial has caused the organic material to become altered by prolonged exposure to temperatures of probably 100°C, producing lower molecular weight, chiefly branched-chain hydrocarbons. The acyclic hydrocarbons that are predominant in the Durham and Nottinghamshire samples are absent from these samples. Triterpanes and steranes are absent and have possibly been degraded, or aromatised and incorporated within the kerogen.

FATTY ACIDS IN MARL SLATE AND ITS LATERAL EQUIVALENTS

In comparison to Green River Shale it was found that Marl Slate contained very low amounts (< 10 p.p.m.) of fatty acids per unit

weight of shale. These acids were not present in detectable amounts in the solvent extracts but were only obtained by acid demineralisation of the samples, followed by saponification of the kerogen.

The fatty acids were extracted from saponified solutions and purified by preparative thin layer chromatography (t.l.c.) on Kieselgel G containing 10% potassium hydroxide. After methylation and column chromatography, the aliphatic esters obtained were analysed by t.l.c., i.r. and g.c. Tentative identification of long chain esters in the shales was obtained by comparing their t.l.c. behaviour and their i.r. spectra with those of authentic esters.

Gas liquid chromatography of the fatty acid methyl esters on 20ft columns containing OV-1, and comparison with authentic esters, revealed the presence of long chain alkanoic acids. The Marl Slate (Downhill) sample had a total fatty acid content of 6.6 p.p.m. per unit weight of shale.

The gas chromatogram, Fig. 19, shows a homologous series of normal alkanoic acids ranging from $\underline{n}C_{10}$ - $\underline{n}C_{26}$. (The fatty acid esters were shown to be saturated by i.r. and argentaceous t.l.c.). It is evident that there is a slight even/odd predominance of these acids and that they have a maximum concentration of $\underline{n}C_{16}$ and $\underline{n}C_{18}$. This is in contrast to the normal alkanes of this sediment whose gas chromatograms show a smooth envelope of peaks. The acyclic isoprenoid fatty acids i.e. C_{13}, C_{14}, C_{15}, C_{16}, C_{18}, C_{19} (pristanic) and C_{20} (phytanic) acids were tentatively identified by comparing the retention indices of some synthetic isoprenoid acids, and those from Green River Shale[91], with those present in the Marl Slate (Downhill) sample, Table 6. The C_{21} acid was also tentatively identified from its retention index on OV-1.

As in the Green River Shale, the dominant isoprenoid acids are phytanic and pristanic acid. The distributions of the acids in Green River Shale and Marl Slate are remarkably similar and suggests that

Fig. 19

Fatty acid methyl esters in the Marl Slate (Downhill)

Chromatographic conditions: see Fig. 7.

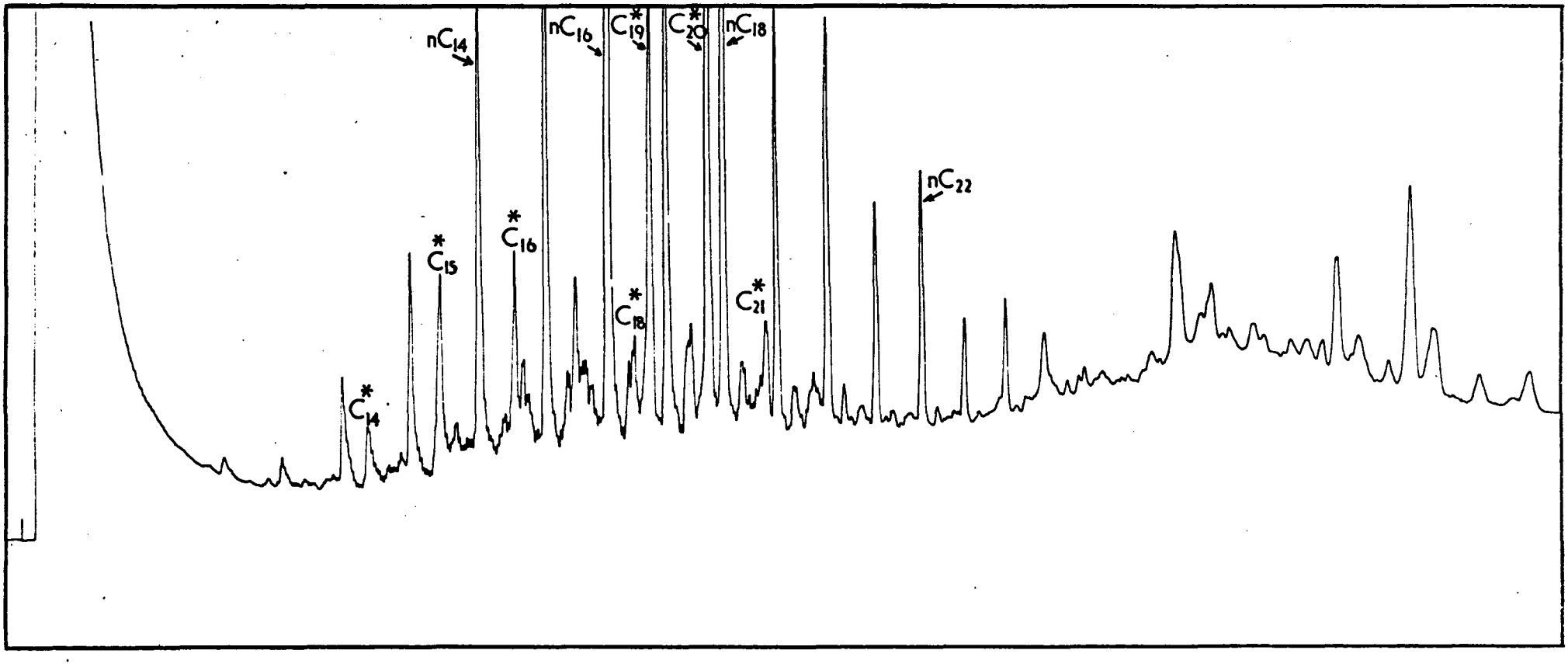

nC14
nC16
C*19
C*20
nC18
nC22
C*14
C*15
C*16
C*18
C*21

TABLE 6. RETENTION INDICES OF GREEN RIVER SHALE AND MARL SLATE ACYCLIC ISOPRENOID ACID METHYL ESTERS ON OV-1.

FATTY ACID	GREEN RIVER SHALE (E.C.L. VALUE)*	MARL SLATE (E.C.L. VALUE)*
C_{14}(2,6,10, trimethyl undecanoic acid-methyl ester)	-	12.40
C_{15}(2,6,10, trimethyl dodecanoic acid-methyl ester)	13.50	13.48
C_{16}(2,6,10 trimethyl tridecanoic acid-methyl ester)	14.50	14.50
C_{17}(2,6,10 trimethyl tetradecanoic acid-methyl ester)	15.50	15.49
C_{18}(2,6,10 trimethyl pentadecanoic acid-methyl ester)	-	16.45
C_{19}(2,6,10,14 tetramethyl pentadecanoic acid-methyl ester)	16.71	16.71
C_{20}(2,6,10,14 tetramethyl hexadecanoic acid-methyl ester)	17.75	17.74
C_{21}(2,6,10,14 tetra methyl heptadecanoic acid-methyl ester)	18.75	18.77

* Values obtained by interpolation between the retention indices of the normal acids in programmed GC analysis.

similar precursor material was present in these depositional environments. It is evident that the relative concentration of high molecular weight (probably triterpenoid) acids is greater, and more complex, in the Marl Slate acids than in those from Green River Shale. However, it should be noted that the concentration of total acids in the Marl Slate is about two orders of magnitude less than those present in the Green River Shale. Both shales are very rich in saturated alkanes and it appears likely that the low level of fatty acids in the Marl Slate is due to a much longer period of burial. It is possible that a process similar to that postulated by Jurg and Eisma[34] and by Cooper and Bray[33] has occurred which caused decarboxylation of the fatty acids. However, if this is correct, it must occur with the fatty acids bound as esters to the kerogen and not by the simple decarboxylation of free fatty acids as postulated by the above authors.

Interestingly, the disappearance of long chain fatty acid esters in coals has also been noted to occur with increasing coalification and to result in the formation of alkanes bearing no odd/even predominance[92,93]. It is evident therefore that the decrease in fatty acid content with age of the sediment can be associated with the disappearance of the n-alkane odd/even predominance. This may be considered to be a "swamping" of the original odd/even predominance with alkanes generated from normal fatty acids. This generation of hydrocarbons may occur, not by the decarboxylation of free fatty acids in the soluble organic material but, by decarboxylation of esters bonded to kerogen.

The origin of these acyclic isoprenoid acids within the sedimentary environment is difficult to elucidate. Phytanic acid could possibly be derived from phytol. It has been shown[94] that phytol can be reduced to dihydrophytol. Oxidation of this could yield the C_{20} acid but it is difficult to understand how conditions within the sediment, during diagenesis, could change from reducing to oxidising. The fatty acids in Green River Shale, if not present in the precursor

organic material must have been generated during a period of about 50-60 million years. Presumably these fatty acids will disappear ultimately due to geothermal decarboxylation or reduction. Bacterial oxidation of the acyclic isoprenoid hydrocarbons appears unlikely since bacterial activity ceases very early in the accumulating sediment at a depth of only several feet. Bacterial oxidation could occur at the outcrop, but most samples collected are unweathered and it would take considerable amounts of bacteria to generate the quantities of fatty acids present in the Green River Shale. Also bacterial oxidations of n-alkanes of ancient sediments would not yield the marked even/odd predominance of the n-fatty acids in the Green River Shale and Marl Slate. Thus although it is possible to show the gradual disappearance of kerogen bound fatty acids with time, and even/odd ratios approaching unity, it is not possible to show how these fatty acids originated in the sediment. Early in the deposition of the sediments unsaturated fatty acids disappear, probably by hydrogenation to saturated fatty acids or by polymerisation reactions involving either autoxidation of mono-enes, dienes or polyunsaturated acids or Diels Alder type addition reactions[59]. These polymers presumably become incorporated within the kerogen which itself is also undergoing formation. For a clearer understanding of these reactions it would be useful to conduct experiments on core samples obtained from present day sediments which have formed under conditions analogous to those of the Green River Shale, (e.g. Caspian Sea). By analysis of the lipids of the organisms which contribute to this sediment it should be possible to understand the factors involved which remove the fatty acids, in an extractable form, from the recently formed sediment[95,96].

(c) CAITHNESS BITUMINOUS SHALES

Analyses of the lipids, present in the more carbonaceous samples of this area, were undertaken in order to compare the types and

distributions of hydrocarbons and fatty acids found with those occurring in younger sediments which were deposited under similar conditions e.g. Green River Shale (60 x 10^6 years ago) the Scottish oil shales of the Lower Carboniferous age (ca. 300 x 10^6 years ago)[13], and the Marl Slate of Permian age (ca. 230 x 10^6 years ago).

Two Caithness samples were studied and analysed for saturated hydrocarbons, fatty acids and porphyrins. A black bituminous shale (Sample A) and a shale from the Wick Flagstone Series (Sample B) were extracted with benzene/methanol to yield 1,400 p.p.m. and 800 p.p.m. of soluble organic material respectively. Both of these extracts were light yellow-brown oils and they were soluble in petroleum ether, which showed that they contained no asphaltene fraction. (The asphaltene fraction is defined as the part of the benzene soluble extract of a petroleum or sediment which is insoluble in hexane.

Visible light spectroscopic analysis of these extracts showed that there was no evidence for the presence of metalloporphyrins or free porphyrins above the detection limits employed (0.01 p.p.m.).

HYDROCARBONS IN THE CAITHNESS SHALES

Each of the sample extracts contained unusually high concentrations of saturated hydrocarbons. The organic extracts of samples A and B consisted of 63.5% and 22.5% respectively of saturated hydrocarbons. The hydrocarbon fractions were obtained by chromatography of these extracts on alumina and they were separated into normal and branched-cyclic alkanes using 5A molecular sieve. The results are shown in Table 7. It was noted that 5A molecular sieving was not quantitative and that the concentrations quoted for the normal alkanes in each sample are low.

Alkane fractions of samples A and B were examined by gas chromatography. The gas chromatogram of the total alkanes from Sample A, Fig. 20, shows a complex mixture of hydrocarbons ranging from about

$\underline{n}C_{11}$ to $\underline{n}C_{35}$. The normal alkane peaks are shown superposed on an unresolved hump of hydrocarbons. The gas chromatogram of the branched-cyclic fraction also shows a complex hump with the isprenoid hydrocarbons, marked in Fig. 20, superposed. There is much material in the $\underline{n}C_{26}$ - $\underline{n}C_{30}$ region of the chromatogram which could possibly be due to the presence of steranes and triterpanes in the hydrocarbon fraction. Lack of time and mass spectrometric facilities did not permit an examination of these higher molecular weight hydrocarbons. Although the mixture is complex the C_{18} - C_{20} isoprenoid hydrocarbons are prominent in the gas chromatogram. This is partial evidence to support a biogenic origin of these hydrocarbons and it is in accordance with Calvin's observations[39] of hydrocarbons in ancient sediments.

The gas chromatogram of the normal alkane fraction shows a bimodal distribution with envelope maxima at $\underline{n}C_{19}$ and $\underline{n}C_{24}$; there is no odd/even predominance in the range $\underline{n}C_{15}$ - $\underline{n}C_{35}$ present in the total alkanes. The normal alkanes occur in a molecular weight range, lower than that normally associated with higher plants, and it suggests that the contribution of alkanes from higher plants is minor.

In sample B, however, the gas chromatograms of the total, branched-cyclic and normal hydrocarbons Fig. 21 shows them to be much less complex and the ratio of resolved to unresolved components is much greater. Most of the hydrocarbons are in the $\underline{n}C_{12}$ to $\underline{n}C_{24}$ region with little evidence of material above $\underline{n}C_{24}$. Once again there is no odd/even predominance of normal alkanes in the range $\underline{n}C_{13}$ to $\underline{n}C_{24}$.

The C_{13} - C_{20} isoprenoid hydrocarbons were tentatively identified by comparing their retention indices with those of Marl Slate isoprenoid hydrocarbons which had been previously characterised by mass spectrometry. The retention indices of the Caithness isoprenoid alkanes were measured on a silicone gum (OV-1) liquid phase and are shown in Table 8. The C_{16} and C_{18} isoprenoid hydrocarbons are present in

Fig. 20

Saturated hydrocarbons in the Caithness shale (Sample A)

A. Total alkanes

Chromatographic conditions: see Fig. 7.

B. Branched-cyclic alkanes

Chromatographic conditions: as above.

C. Normal alkanes

Chromatographic conditions: as above except that the oven was programmed from 100° - 290°C.

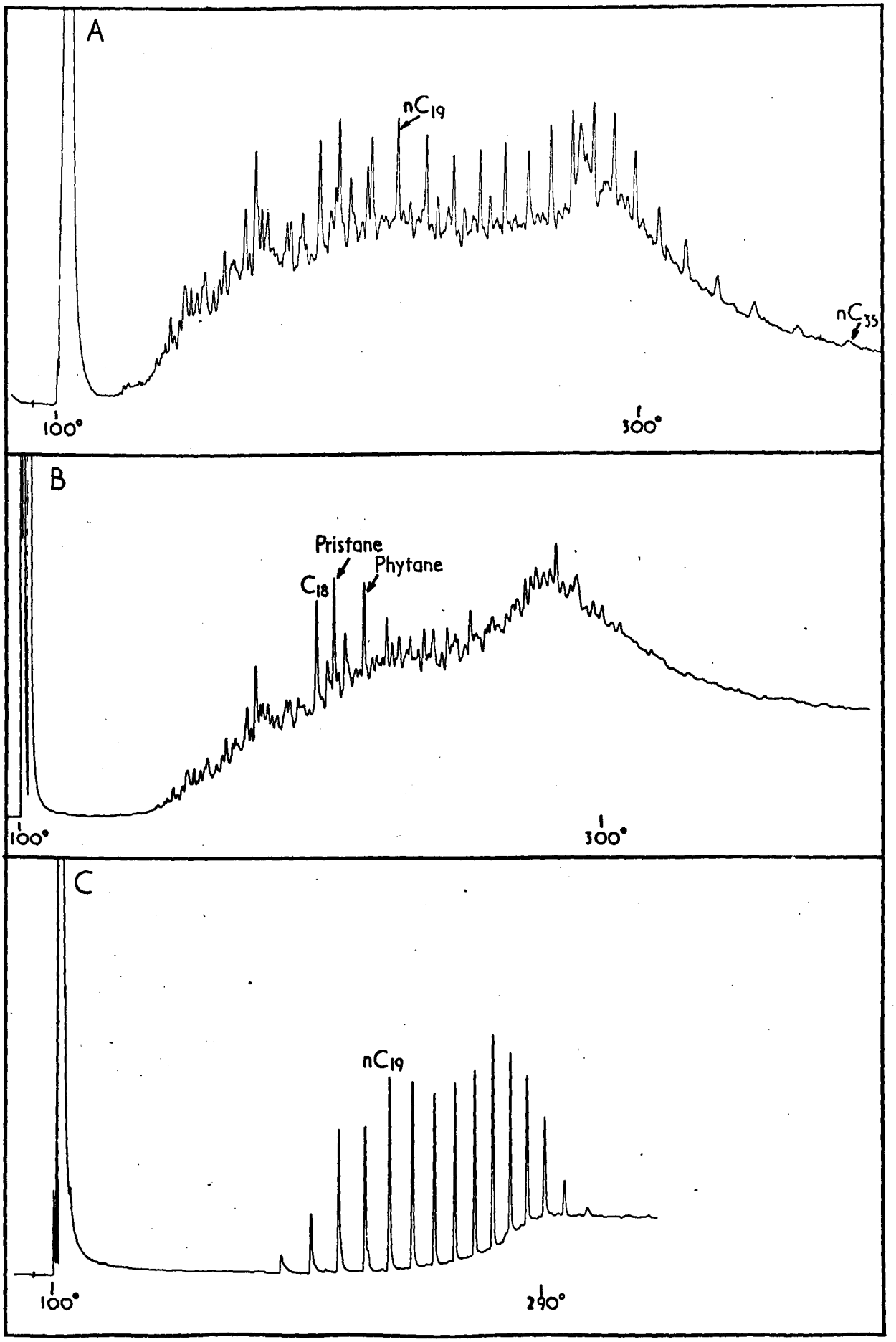

A
nC19
nC35
100°
300°
B
Pristane
Phytane
C18
100°
300°
C
nC19
100°
290°

Fig. 21

Saturated hydrocarbons in the Caithness shale, (Sample B)

A. Total alkanes

Chromatographic conditions: see Fig. 7.

B. Branched-cyclic alkanes

Chromatographic conditions: as above.

C. Normal alkanes

Chromatographic conditions: as above.

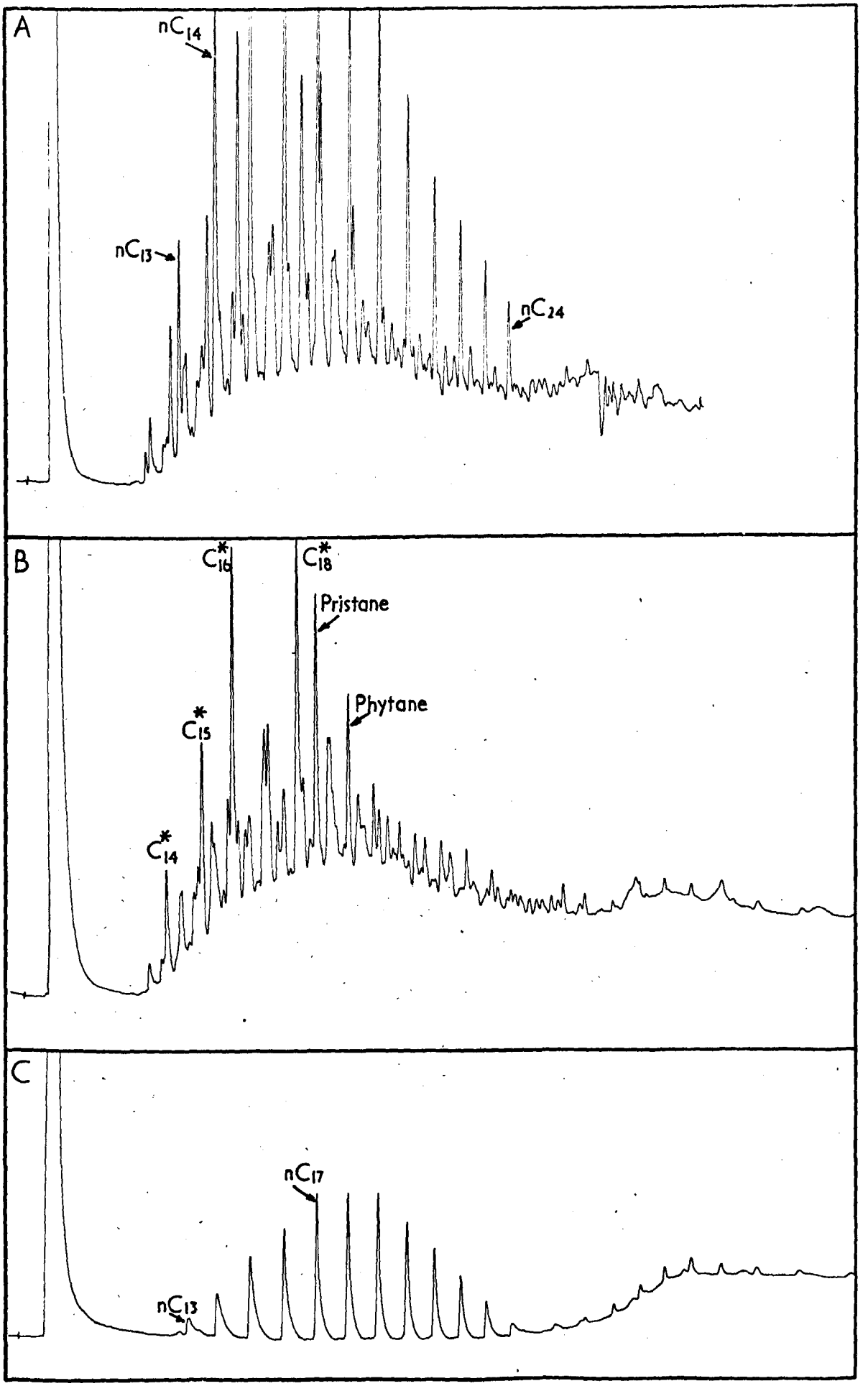

A
nC14
nC13
nC24
B
C*16
C*18
Pristane
Phytane
C*15
C*14
C
nC17
nC13

TABLE 7. CONCENTRATIONS OF ORGANIC MATERIAL IN THE CAITHNESS SAMPLES

	SAMPLE A. (185g)	SAMPLE B. (150g)
SOLUBLE ORGANIC MATERIAL	261 mg (1,410 p.p.m.)	121 mg. (807 p.p.m.)
TOTAL ALKANES	165 mg (890 ")	27.1 mg (181 ")
BRANCHED/CYCLIC ALKANES	154 mg (830 ")	9.5 mg (63 ")
NORMAL ALKANES	4.7mg(25 ")	13.3 mg (89 ")

TABLE 8. KOVATS RETENTION INDICES OF MARL SLATE AND CAITHNESS ISOPRENOID HYDROCARBONS ON OV-1

ISOPRENOID HYDROCARBON	MARL SLATE	CAITHNESS (SAMPLE B)	AUTHENTIC STANDARDS
C_{13}	1215	1215	
C_{14}	1275	1275	
C_{15}	1379	1380	1376
C_{16}	1465	1465	1464
C_{18}	1652	1652	1651
C_{19}	1709	1709	1819

greatest concentration although the concentration of the C_{14}, C_{19} and C_{20} isoprenoid hydrocarbons is considerable. The gas chromatogram of the branched-cyclic fraction is substantially like that of the Marl Slate and Green River Shale except for the predominance of the C_{16} and C_{18} isoprenoid hydrocarbons. It is noteworthy that there are no hydrocarbons in the C_{30} region.

Sample A is from an area in which post-depositional igneous activity took place. The occurrence of igneous dykes close to the locality of the bituminous shale suggests that the sediment could have been exposed to increased temperatures. The bitumen, which is very similar to ozocerite, is also found injected into veins and fissures and is fluid when freshly exposed. This is suggestive of hydrothermal re-deposition and in fact these bituminous samples show marked similarities with elaterite occurrences in Derbyshire, previously described by Mueller[96], Ponnamperuma[16] and Pering et al[15]. The latter authors have argued on the basis of the complexity of the saturated hydrocarbons in the Derbyshire elaterites that these hydrocarbon deposits are abiogenic in origin. The writer believes that elevated temperatures in the Caithness area, associated with hydrothermal mineralisation, may be responsible for causing thermal degradation of the kerogen in these sediments to yield a high saturated hydrocarbon/organic extract ratio. It has been estimated* that sediments 40 ft. away from an igneous dyke 20ft in thickness e.g. sample A, could have been subjected to temperatures of 150°C - 190°C for a duration of 50-80 yr.

Sample B, obtained from an area relatively free from igneous activity showed a less complex hydrocarbon pattern and a lower percentage (22.5%) of saturated hydrocarbons than sample A. Nevertheless, hydrocarbons still account for a very large part of the extractable material. Although only two samples were studied, it is

* Personal communication Dr. B.S. Cooper

possible that the differences represent the effect of elevated temperatures on original biogenic material. Branched chains in the kerogens of these samples would be thermally more labile than unbranched chains and thus one would expect to find branched hydrocarbons in greater abundance than normal chains. The result of an increasing complexity of branched chain hydrocarbons i.e. an increase in the number of isomers would give the appearance of an abiogenically synthesised hydrocarbon mixture and tend to mask an original biogenic distribution of precursor organic material.

FATTY ACIDS IN THE CAITHNESS SHALES

Saponification of the shale samples, followed by acidification and extraction, afforded a fatty acid fraction which was esterified with BF_3/MeOH. After characterisation by t.l.c. and i.r. spectrometry the acid esters were analysed by gas liquid chromatography. The procedure adopted was analogous to that used for the Marl Slate samples.

The esters were analysed by g.c. using a silicone gum liquid phase (OV-1), and authentic normal fatty acid esters were coinjected with the extracted esters in order to characterise individual peaks. It was not possible to determine whether the branched acids present were the iso- or anteiso- acids, since the column did not have sufficient resolution (13,000 theoretical plates) to separate these positional isomers. The large peak eluting after the nC16 methyl ester has an E.C.L. value close to that of methyl pristanate, Table 6, and may indeed be this compound; the other branched acids however do not have E.C.L. values similar to the other isoprenoid acids.

The gas chromatogram of sample B, acid esters, shown in Fig. 22 is a temperature programmed analysis of the methyl esters of the extracted fatty acids. A "blank" chromatogram showing the amount of liquid phase bleed at 300°C is also provided. Fatty acid esters

Fig. 22

Fatty acid esters in the Caithness shale (Sample B)

Chromatographic conditions: see Fig. 7.

nC20
nC18
nC17
iC17
iC16
nC16
nC15
iC15
nC14
iC14

ranging from dodecanoic acid ($C_{12:0}$) acid are present with minor amounts up to nC_{28}. The predominant normal acids are myristic, ($C_{14:0}$) palmitic ($C_{16:0}$) and stearic acids ($C_{18:0}$). Branched acids are also evident and are identified as the iso- or anteiso- C_{14}, C_{15}, C_{16} and C_{17} acids. The absence of unsaturated fatty acids in this acid extract was shown by i.r. analysis and argentaceous t.l.c.

Sample A contained no detectable fatty acids. This analysis established the fact that contamination with fatty acids of laboratory origin did not occur above detectable limits. The gas chromatogram of sample B esters contains a large proportion of branched-chain esters. Similar results from Precambrian rocks have been obtained by Van Hoeven et al[78], who have suggested that the absence of unsaturated fatty acids in their sample extracts indicates that recent bacterial contamination has not occurred. Recently, Smith et al[79] have analysed the lipids present in extracts of Precambrian and Phanerozoic cherts. The concentrations of hydrocarbons and fatty acids found were much lower than those found by previous workers, and were shown to be more concentrated on exposed surfaces of the chert. They have criticised the work of Van Hoeven et al[78] and believe that the esters examined were the result of post-depositional contamination. This contamination is believed to have occurred in situ at the rock outcrop.

The strong predominance of fatty acids with even carbon numbers in the Caithness shale resembles the lipid fatty acid patterns in extant flora and fauna. Substantial amounts of branched fatty acids suggests that they were derived from the lipids of micro-organisms, e.g. bacteria. It is possible that bacterial contamination of the rock outcrop by percolating groundwater may have occurred. Although unsaturated fatty acids might have been expected to occur as contaminants, Smith et al[79] have shown that microgram quantities of unsaturated acids in their extracts rapidly disappeared within several days of extraction of their chert samples. Thus, the presence of fatty acids in the

Caithness sample may be the result of post-depositional contamination. The absence of detectable amounts of fatty acids in the other Caithness sediment provides a check that contamination by C_{14}, C_{16} and C_{18} acids did not occur in the laboratory.

CHAPTER V

PORPHYRINS IN THE MARL SLATE

(a) INTRODUCTION

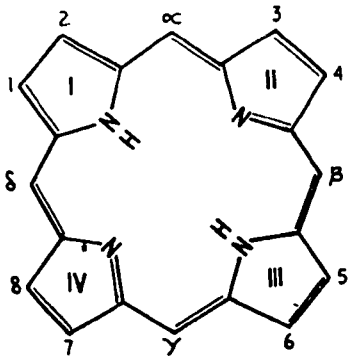
Tetrapyrrole pigments chelated with iron or magnesium play an important role in nature. These pigments are involved in photosynthesis, nitrogen fixation in plants, oxygen transport in respiratory organisms and they form the prosthetic groups of the haem enzymes which include the cytochromes, peroxidases and catalases[97].

Basically there are two different structural types of tetrapyrrole pigments, chlorophyll and haemin types, based on a parent nucleus, porphin. These are represented in Figs. 23 and 24. Porphyrins are derivatives of porphin in which the β-carbon atoms 1-8, of the four pyrrole nucleii, carry substituents other than hydrogen atoms.

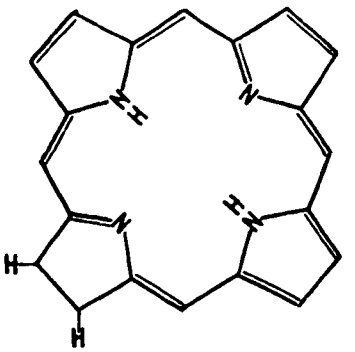
Reduction of porphin at the 7,8 position in ring IV results in the formation of a chlorin Fig. 23, (dihydrophrphyrin). Further reduction is possible to a tetrahydroporphyrin (Fig. 23), respresentative of the nucleus found in bacteriochlorophylls. A structure of the chlorophyll type is represented by a chlorin nucleus with an isocyclic ring linking the carbon atoms at position 6 in ring III to the methine bridge carbon atom at the γ position of the chlorin nucleus. The haemin structural type is more closely related to the basic porphin skeleton, differing only in the occurrence of acid, alkyl or alkenyl substituents, on the four pyrrole nucleii.

All naturally occurring porphyrins and chlorins contain carboxyl substituents and a great many possess vinyl and aldehyde substituents. In photosynthetic organisms a number of types of chlorophylls are present; chlorophylls (a, b, c, d and e), bacteriochlorophylls (a, b and c) and bacterioviridins. Chlorophyll a is the most abundant and is generally found in association with chlorophyll b in higher

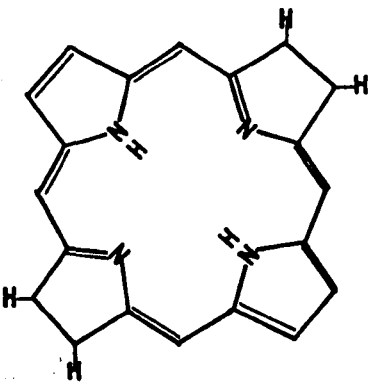
Figure 23



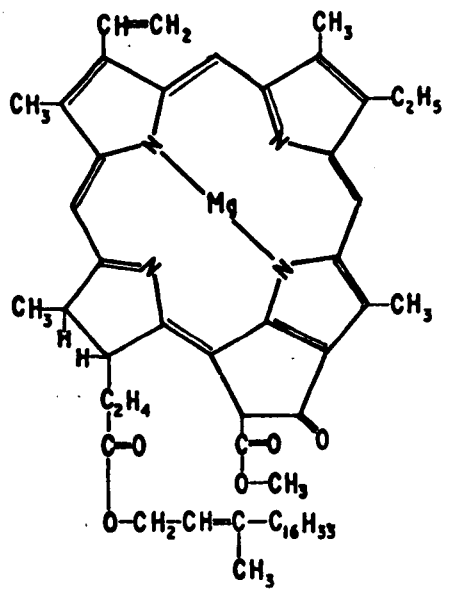
PORPHIN



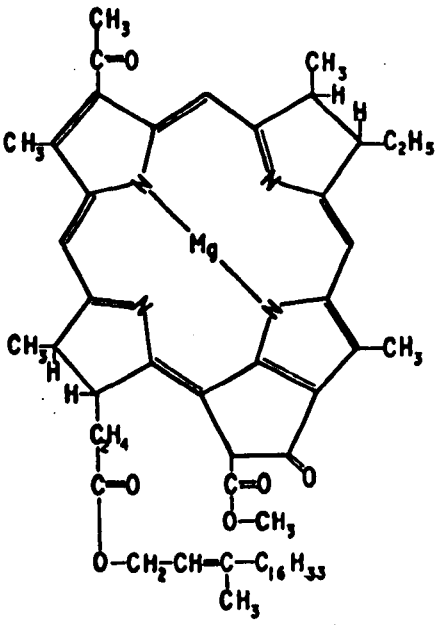
CHLORIN



TETRAHYDROPORPHIN

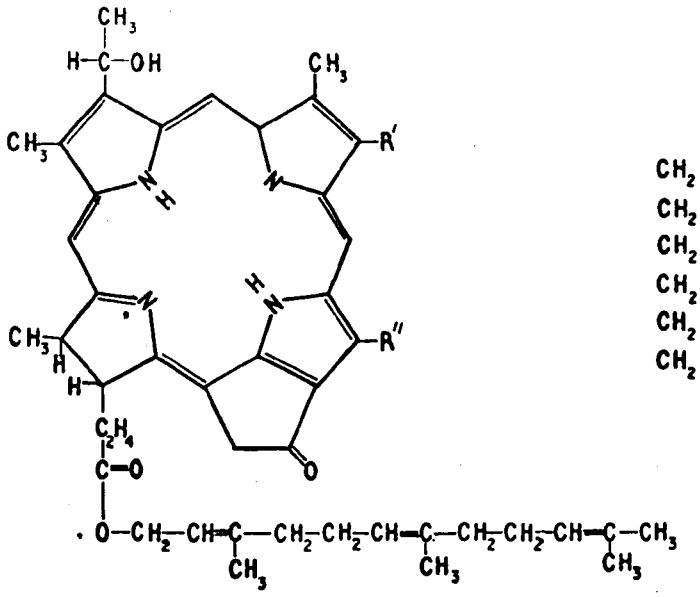


CHLOROPHYLL a



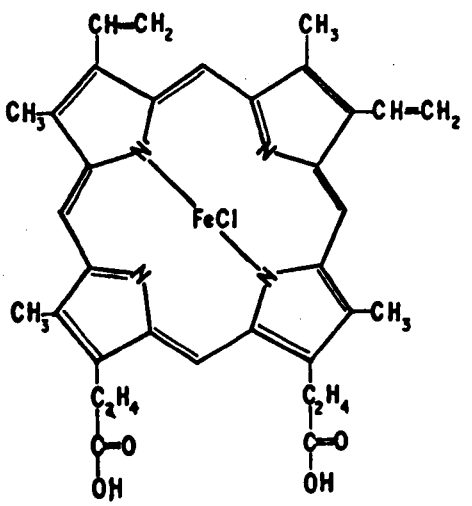
BACTERIOCHLOROPHYLL a

Figure 24

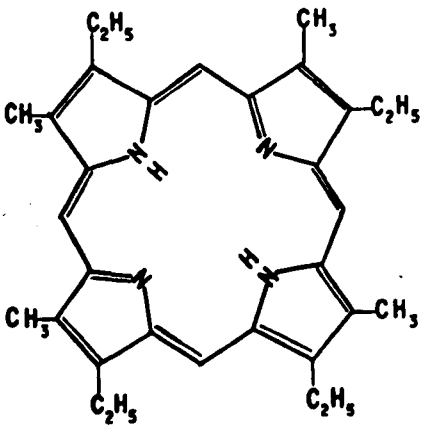


R'	R''	CARBON No.
$CH_2CH(CH_3)_2$	CH_2CH_3	35
$CH_2CH_2CH_3$	CH_2CH_3	34
$CH_2CH(CH_3)_2$	CH_3	34
CH_2CH_3	CH_2CH_3	33
$CH_2CH_2CH_3$	CH_3	33
CH_2CH_3	CH_3	32

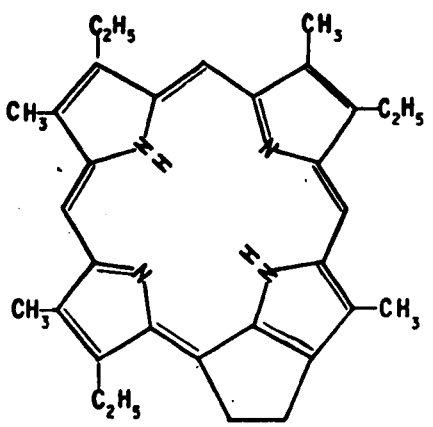
CHLOROBIUM CHLOROPHYLLS



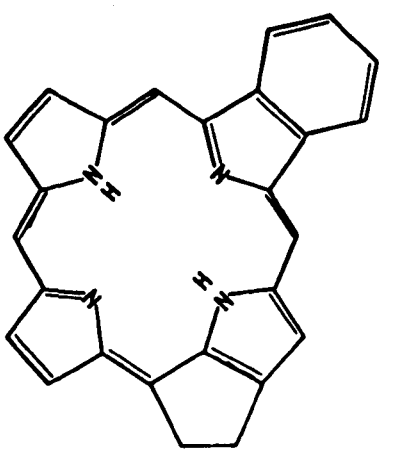
HAEMIN



MESOETIOPORPHYRIN



DEOXOPHYLLOERYTHROETIOPORHYRIN



MONOCYCLANOBENZPORPHIN

plants and algae. Chlorophylls c, d and e are found in algae and are associated with chlorophyll a. The bacteriochlorophylls are present in purple and brown bacteria. Bacterioviridins otherwise referred to as chlorobium chlorophylls are present in several green photosynthetic bacteria and are unusual in that farnesol, and not phytol, is the esterifying alcohol of the propionic acid substituent in ring IV[98].

From these numerous porphyrins, chlorins and tetrahydroporphyrins occurring in biological systems only a few persist in the sedimentary environment. The original precursor pigments dispersed with other organic debris have undergone a series of alterations involving the reduction of carbonyl groups (carboxyl and aldehyde), reduction of vinyl groups (Treibs mechanism), decarboxylation, and possibly alkylation and transalkylation of the peripheral positions of the tetrapyrrole ring[99,100,101]. Cooper and Bray's[33] proposal that fatty acids may decarboxylate, in the geological environment, through an acylate radical to an alkyl radical which may then abstract hydrogen to form an alkane can possibly be applied to the diagenesis of natural porphyrins. Although this hypothesis may not account for petroleum porphyrin (petroporphyrin) carbon skeletons containing more than 32 carbon atoms (carbon number of the haemin molecule) it is possible that other mechanisms operating during diagenesis can account for the petroporphyrins containing up to 36 carbon atoms. Baker et al[102] overcame this difficulty by proposing a diagenetic transalkylation mechanism to account for the higher molecular weight petroporphyrins. An alternative possibility for the occurrence of these higher molecular weight porphyrins arose from the finding of Holt[103] et al who showed that porphyrin skeletons of bacterioviridins contained up to 35 carbon atoms. By applying the Treibs mechanisms, i.e. reduction and decarboxylation, to these chlorobium chlorophylls it is possible

to postulate a distribution of porphyrins in the range C_{32}-C_{35}, similar to that found in petroleums by Morandi and Jensen[104] and by Baker et al[102].

Recently, Baker's suggestion has gained support from the work done by Hoering[105] on the thermal treatment of recent marine sediments. Chlorophyll was the major pigment present in the sediments and after heat treatment at 225°C for several days the porphyrins generated were a complex mixture of homologues suggesting that reduction, alkylation and transalkylation of the peripheral substituents and positions had occurred.

The two most common porphyrins occurring in the geologic environment are deoxophylloerythroetioporphyrin (DPEP) and mesoetioporphyrin (MEP) both of which are shown in Fig. 24. When these compounds occur they usually constitute one member of a homologous series differing in the number of methylene units substituted on the porphyrin peripheral positions; they are believed to be derived from chlorophyll and haemin type precursors. This suggestion was first made by Treibs[106], and the presence in petroleum of either or both of these petroporphyrins is generally thought to support a plant and/or animal organic accumulation from which the petroleum was derived. This has proved a potential tool for the organic geochemist in establishing both a biogenic origin for petroleum, and petroleum-like substances, and the type of organic material (plant or animal) which was altered, by diagenetic processes to form petroleum.

Hoering's work[105] illustrates the potential of heating sediments in order to understand and relate the mechanisms occurring in the laboratory to those that could possibly occur over geologically prolonged periods. By producing data by which DPEP and MEP can both be derived from chlorophyll alone it may well invalidate many previous conclusions which considered that DPEP and MEP were derived from chlorophyll and haemin pigments respectively.

Porphyrins are perhaps the first of the organic constituents of petroleums and ancient sediments that were attributed to biogenic sources.

They are present in varying concentrations (1 to 1000 p.p.m.) in petroleums and sedimentary rocks ranging in age from early Precambrian to Recent. Barghoorn et al.[107] have described the presence of vanadyl porphyrins in the Nonesuch formation (1×10^9 yrs). They have used silica gel column chromatography and u.v. spectrophotometry to detect these porphyrins and they have suggested that other porphyrins, containing carbonyl groups, are present. They have suggested that the porphyrins in the Nonesuch formations constitute the oldest known direct evidence of photosynthetic mechanisms on our planet. Hodgson and Baker[108] have identified a carboxylated vanadyl porphyrin, present in very low concentration (0.011 p.p.m.) from a sample of the Orgueil carbonaceous chondrite. More recent work has shown the presence of porphyrins in other meteorites[109]. Reports on the occurrence of indigenous porphyrins in Precambrian rocks older than 3.2×10^9 years may yet suggest however the genesis of these porphyrins by abiogenetic processes. It is possible that before life arose upon the primitive earth (now thought to have formed about 4.75×10^9 years ago) abiogenetic processes produced organic compounds similar to biochemicals present in contemporary organisms. Thus the occurrence of the so-called "biological markers" may not be indicative of the presence of life in these former times[39].

Hodgson and Baker[110] have recently conducted "primitive atmosphere experiments" under simulated geochemical conditions and have been able to synthesise copper porphyrins in microgram quantities from pyrrole, paraformaldehyde and copper salts. Electric discharge experiments on methane, ammonia and water systems have similarly synthesised porphyrins, among other reaction products[111].

Conditions under which abiogenesis of these organic molecules could have taken place are believed to have prevailed until some 1.8×10^9 years ago (Urey level) before the oxygen content of the atmosphere began to increase above 0.1% as a result of photosynthesis[112]. Before the change to oxygenic conditions the presence of organic compounds

accumulating in sedimentary environments could be the combined effect of biogenic and abiogenic processes. The occurrence of porphyrins in the oldest rocks known $> 3.2 \times 10^9$ yrs, may suggest that biological markers need not necessarily demand a biogenic origin as is inferred by Barghoorn et al[107].

An excellent review of literature[113], prior to 1964, is available on the organic geochemistry of porphyrins, dihydroporphyrins (chlorins), and their isolation by chromatographic techniques (excluding g.c.). Characterisation of porphyrins and chlorins by u.v. spectrophotometry has been discussed in detail[114]. Some authors have also elaborated on the possible mechanisms by which chlorophyll a and haemin could be degraded, within the sedimentary environment, to DPEP[115].

Since the above review appeared many other references are available on improved chromatographic and mass spectrometric techniques for isolating and characterising petroporphyrins.

It is considered that a review of the literature prior to 1967 would only duplicate the observations and conclusions expounded in detail by Hodgson et al[113]. Although a general review of the petroporphrin literature has been written since it is pertinent to review the more recent literature which is applicable to the results recorded later in this thesis.

An improved method for demetallation of the porphyrins chelates present in crude oil has been patented by Erdman[117]. This has marked advantages over the digestion of the crude oil by acetic acid-hydrobromic acid mixtures, originally used by Treibs[118]. The method achieves the cleavage of the chelating metal by heating with an alkyl sulphonic acid at 10°C to 250°C (generally 50°C) for half an hour with stirring. The procedure is quantitative and more rapid than former methods. Although a demetallation procedure does not allow the chelating metal to be determined, it is a valuable technique for increasing the volatility of the porphyrins prior to m.s. identification. A demetallation procedure

is, of course, necessary before substitution of the chelating atom by a hexacoordinate atom (e.g. silicon) suitable for the formation of volatile derivatives of porphyrins for examination by g.c. [119,120,121].

The most detailed knowledge of the structures of components of the porphyrin fractions of crude oils has been accomplished by mass spectrometry. Morandi and Jensen[104], Baker et al.[102] and more recently Yen[116] have identified the various homologues of MEP, DPEP and aryl porphyrins using high resolution mass spectrometry. The latter workers have employed methane sulphonic acid digestion prior to mass spectral analysis.

Morandi and Jensen[123] have reported the occurrence of porphyrins in retorted oil shale. This work demonstrated that the skeleton of the porphyrin molecules in oil shale can survive retorting temperatures of 900°F and that the character of the shale porphyrins is changed from a phyllo type to an etio type by retorting. They have shown by adsorption chromatography and mass spectrometry that porphyrins in Green River Shale and Wilmington petroleum consist of two homologous series of porphyrins, etio and phyllo types, differing only in the number of methylene substituents (7 to 13 methylene substituents per molecule). The phyllo type predominate and the presence of carboxyl groups in some of the porphyrin molecules was observed.

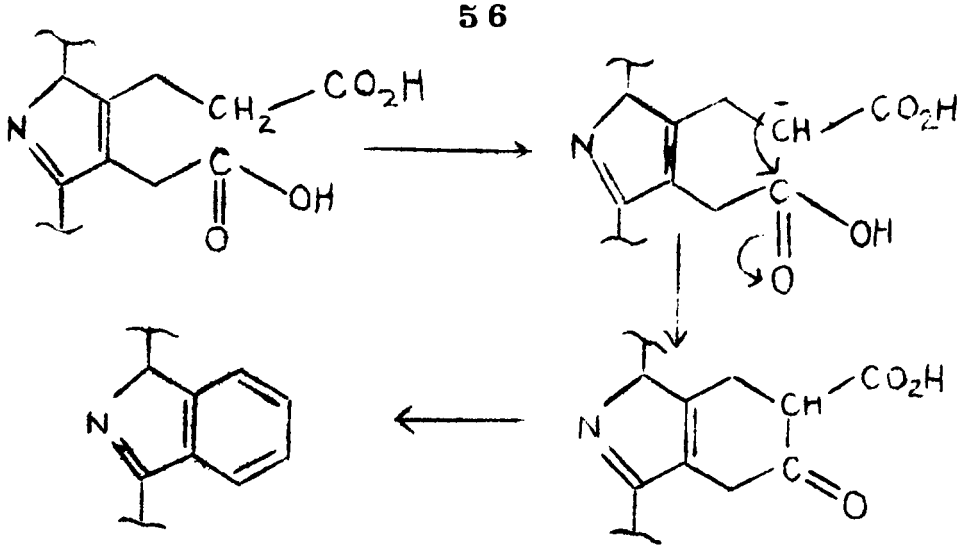
Baker et al. have analysed Green River Shale and a wide range of petroleums and have confirmed the previous workers findings for the distribution of porphyrin homologues in Wilmington petroleum and Green River Shale. They also observed that the ratio of the two series, DPEP/MEP coupled with the molecular weight range, see Fig.26 , varied rather widely with the source; they have suggested that a high DPEP/MEP ratio, associated with a narrow molecular weight range is indicative of a non-marine origin of the petroporphyrins. The authors were also able to provide more information on the structure of rhodo-type porphyrins (e.g. monocyclanobenzporphin, Fig. 24) occurring in minor

concentration in some petroleums. The mass spectrum of Melones petroporphyrins represented a series which had molecular fragment ions 4 and 6 mass units lower than the DPEP series. Visible spectra suggested the presence of an electron withdrawing group and from other data they have proposed an alkyl benzene substituted porphyrin structure which accounts for the 6 mass units deficiency when compared with the MEP series. Similarly they have proposed that a 4 mass units deficiency is due to the presence of a monocyclanoalkylbenzene substituted porphyrin series (e.g. Fig. 24).

Yen and co-workers[122] have studied the vanadyl porphyrin complexes in the asphaltene fraction of petroleum and have similarly observed the above mentioned porphyrins. Mass spectrometric analysis has shown that five series of porphyrins exist in Boscan petroleum asphaltenes and that they differ from the etioporphyrin homologous series (M) by the following mass units:

$$M,\ M-2,\ M-4,\ M-6,\ M+6.$$

For the mass distributions $M^{+}2$, $M^{+}4$, $M^{+}6$, the authors have tabulated the possible porphyrin structures which could be present. Precise mass measured molecular weights of vanadyl porphyrins in the Boscan asphaltene have shown that the porphyrins containing hexahydro structures and more than one benzene substitution are not favoured. The structures which are possible include all possible combinations of any two isocyclic rings, benzene or phenyl and even numbered hydro structures of porphyrin nucleii. The diagenetic processes responsible for the formation of the monobenzporphyrins (rhodo type) may involve a cyclisation between adjacent carboxylic acid or ester substituents (Dieckmann reaction) of a coproporhyrin or uroporphyrin precursor. Decarboxylation followed by reduction and aromatisation may yield a monobenzporphyrin.



Considerable knowledge of the structural types of porphyrins present in the geological environment has been compiled due largely to the application of mass spectrometry. Improved separatory techniques such as g.c. are now being developed and should contribute to the analysis and structural identification of porphyrins. The relative involatility and high molecular weight of both the free porphyrins and their metal chelates has made the g.c. of porphyrins extremely difficult although conventional gas liquid chromatography has been applied to the separation, and identification, of some pyrolysis products of porphyrins and chlorophylls[113].

Corwin et al[124,125,126] have successfully separated a number of porphyrins and their metal chelates by hyperpressure gas chromatography (h.p.g.c.) using dichlorodifluoromethane as carrier gas at pressures up to 3100 p.s.i. Attempts to separate the isomers (II and III) of etioporphyrin were unsuccessful. Columns of higher resolution with different liquid phases may overcome this difficulty.

Boylan and Calvin[119] have synthesised more volatile porphyrin derivatives by preparing silicon complexes. They have prepared and identified a bis (trimethylsiloxy)-Si^{IV} complex using u.v. absorption spectra and mass spectrometry, and reported its successful analysis by g.c. More recently Boylan and Eglinton[121] have extended this technique to the g.c. of petroporphyrins isolated from Boscan petroleum

and Green River Shale. Disadvantages that have arisen from the synthesis of these volatile complexes are - (i) the formation of the silicon complexes is not quantitative, (ii) by products other than the bis(trimethyl siloxy) derivatives are formed, (iii) breakdown of the volatile silicon complexes occurs to a substantial degree on the silanised glass supports of the packed columns and (iv) the volatile silicon complexes are eluted only after repeated injections of the derivatives and consequent overloading of the glass columns.

If it were possible to synthesise other more stable volatile metal complexes of porphyrins, from other octahedral hexaco-ordinate metals atoms, these, if they could be prepared quantitatively, and chromatographed by conventional means, would have decided advantages over h.p.g.c.

Either of these methods should provide a means by which small amounts of petroporphyrins, could be separated and analysed using the combination of g.c.-m.s.

Blumer and Snyder[127], Rimington *et al*[128] and Bachmann *et al*[129] have reported the separation of metalloporphyrins by gel permeation chromatography over alkylated Sephadex (LH-20) and Styragel. Polymeric units of pigments from the Serpiano oil shale extending to high molecular weights (*ca.* 20,000) were obtained from eluates of the porphyrin fraction. The mode of formation of these polymers is not known but the authors tentatively suggest radical coupling of monomer units generated by decarboxylation of carboxylated porphyrins. The precise composition of these polymers is not made clear and it is possible that the complexed material is similar to the protein fragments that Hodgson *et al*[130] have recently shown to be associated with porphyrin extracts of ancient and recent sediments. These authors have identified protein moieties ranging in molecular weight from several thousand to 20,000; they have also analysed the proteins

for their amino acid composition. The abundance of the amino acids in the porphyrin and chlorin hydrolysates was found to be similar to that of ancient sediments suggesting the preservation of peptides originally co-valently bonded to chlorophyll or haem in the source organisms. Increasing evidence indicates that petroporphyrins are derived, to some extent, from resistant chlorins which exist in trace amounts, as non-magnesium metal complexes, in living organisms[113].

The most comprehensive report on the nature of petroporphyrins in various sedimentary environments has recently been published by Hodgson et al[131]. Two hundred and forty nine samples were analysed for their porphyrin, chlorin, polycyclic aromatic and alkane contents. The sediments varied from Recent to middle Precambrian (ca. 2×10^9 yr) in age and consisted of argillaceous, carbonate and arenaceous sediments including various soils and igneous and metamorphic rocks. Using u.v. spectrophotometry, the type of chelating metal (iron, nickel and vanadium) was determined and the concentrations of each of these metal chelates was recorded as parts per million.

The important general conclusions which were obtained may be briefly summarised.

(i) Nickel and vanadyl porphyrins occurred in a wide enough range of samples to indicate an almost ubiquitous occurrence in sedimentary environments.

(ii) Metalloporphyrins occurred preferentially in marine fine-grained clastic rocks ranging from Tertiary to Precambrian in age.

(iii) Metalloporphyrins, in contrast to chlorins, are not common in recent sediments and soils.

(iv) Iron porphyrins were found in environments containing high proportions of other metal porphyrin chelates.

(v) No porphyrins chelated with cobalt, copper or zinc were identified at the level of detection employed.

(vi) There was no apparent relationship between the content of isoprenoid hydrocarbons and the content of total porphyrins or organic matter present in the sediments. Phytane and total porphyrin content show no apparent relationship.

Gransch and Eisma[132] have isolated vanadyl porphyrins chelates from the La Luna (Cretaceous) formation of Western Venezuela, and deduced that the source rocks of the Western Venezuela crude oils could be correlated with this sedimentary formation. A decrease in concentration of porphyrin chelates with depth of burial was apparent and the authors ascribed this to a gradual destruction of porphyrins with increasing temperature. Since the organic carbon, and vanadium content is qualitatively similar throughout the formation, the absence of porphyrins in the deeper sediments appears to indicate a relative instability of the porphyrin structure to prolonged heating in the geologic environment. If this is true it is surprising that porphyrins are found in very ancient sediments e.g. the Nonesuch shale of Precambrian age.

(b) METALLOPORPHYRINS IN THE MARL SLATE

The Marl Slate of County Durham was found to be unusual in that the concentration of metallo-porphyrins was exceptionally high, being more than 1000 p.p.m. per unit weight of soluble organic material. This high concentration of porphyrins warranted an organic geochemical study of the types of porphyrins present and the nature of the chelating metals. No evidence for the presence of chlorins or free porphyrins was obtained and it is interesting to note that Hodgson[131] found that chlorins were characteristic constituents of about 70% and 90% of soils and recent sediments respectively. The presence of chlorins in ancient sedimentary environments was suggested as being indicative of contamination by contemporary plants, soils, and recent sediments. It is important in geochemical analyses to

have samples that have not been contaminated and by studying the different types of organic compounds in these samples possible contamination hazards can be evaluated.

The types and distribution of porphyrins occurring in the Marl Slate have been shown to be similar to those occurring in other ancient sedimentary rocks and it has been possible to suggest the type of original precursors from which they were derived. Also, from the relative amounts of the homologous series of deoxophylloerythroetioporphyrin and mesoetioporphyrins present it has been possible to show a marine origin for the Marl Slate porphyrins. The presence of metallo porphyrins was also established in the Nottinghamshire samples from Kneesall (BQ 7276) and Milton (BK 9825) and these were shown, from their visible light spectra, to be qualitatively similar to those of the Marl Slate of County Durham. There was no evidence of porphyrin chelates in the Kupferschiefer samples from the North Sea area. Gransch and Eisma[132] have ascribed the disappearance of porphyrins in the La Luna formation of Venezuela as due to increasing depth of burial associated with increasing temperature. It is quite possible that porphyrins, originally present in the Kupferschiefer samples have been degraded by thermal action. Hodgson and Baker[128] studied the stability of porphyrin in crude oil and calculated that the activation energy for degradation of the porphyrin nucleus was 52.5k.cals/mole indicating considerable stability. Morandi and Jensen, as noted previously, reported porphyrins in shale oil, retorted from oil shale at 900°F (482°C) which suggests that porphyrins are reasonably stable organic compounds. It would be of some importance to determine the stability of these organometallic complexes <u>in situ</u> so that it would be possible to deduce if the absence of porphyrins in some sedimentary rocks is due to the absence of their precursors in the original organic biomass or due to destruction of the four pyrrolic nucleii by thermal degradation.

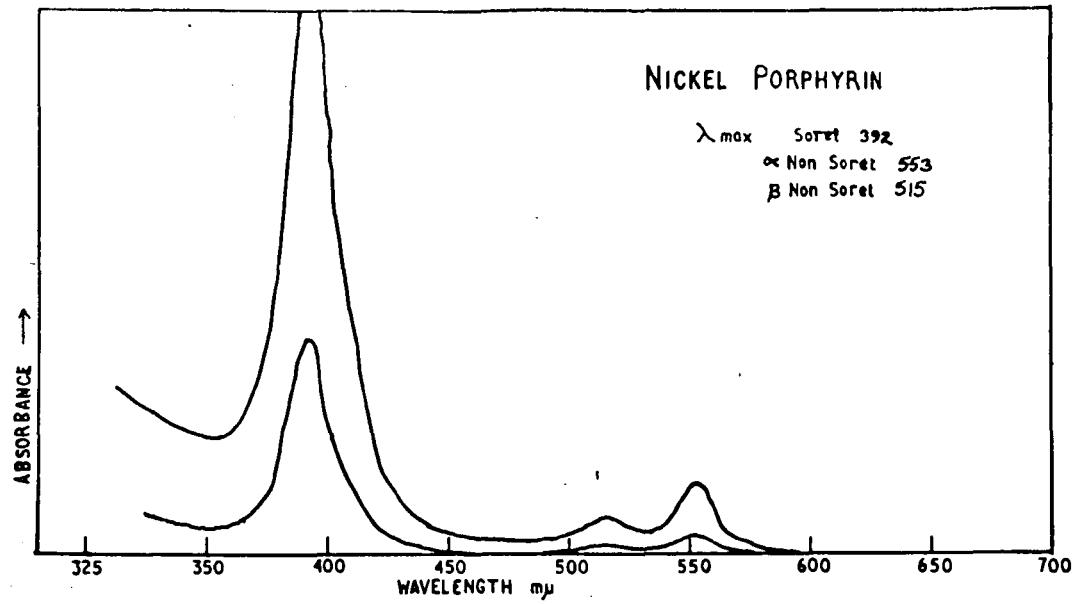
Quantitative results were obtained for the concentration of nickel and vanadyl chelates of the Marl Slate (Downhill) samples. Concentrations were calculated from the absorption of the Soret band near 400 mμ using the extinction coefficient of 3.3×10^5 l/mol/cm measured by Hodgson and Baker[114]. Visible spectra were recorded of the porphyrins in chloroform solutions.

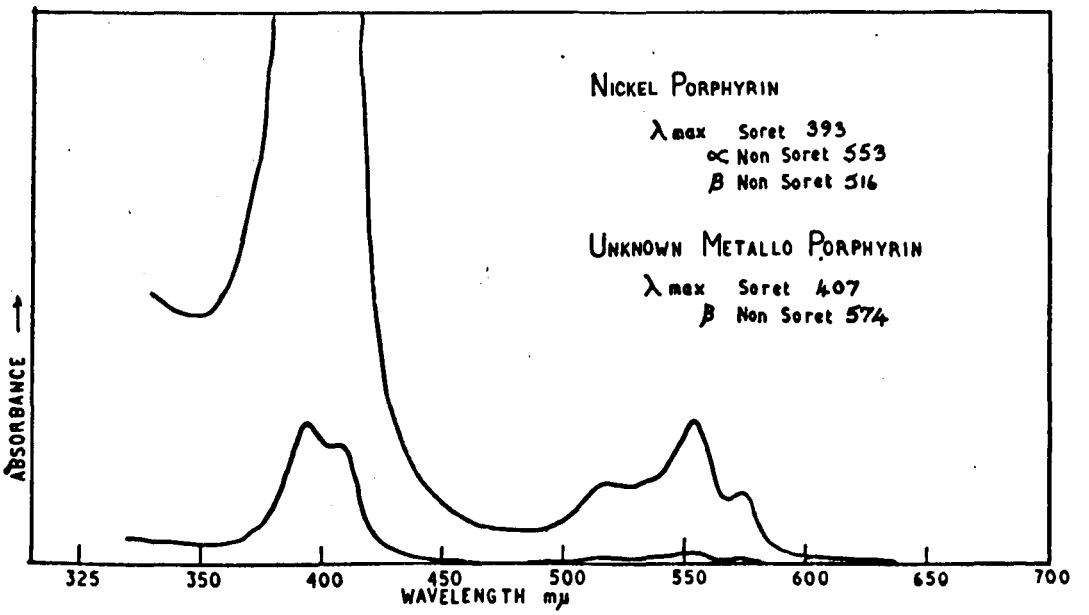
Preliminary crude separations of the metalloporphyrins, obtained by solvent extraction and acid demineralisation of the shale, were carried out using alumina column chromatography. This was necessary to prevent interference caused by the alkanes and aromatic fractions present. The metallo-porphyrins were recovered from the benzene–10% methanol eluate and were further fractionated into their nickel and vanadium chelates by chromatography on silica gel, using essentially the method of Hodgson et al[131]. The metallo-porphyrins were also analysed by thin layer chromatography on Kieselgel G plates using benzene–10% chloroform as developer and it was apparent that better separation of the metallo-porphyrins from strongly fluorescing aromatic compounds could be achieved by this method.

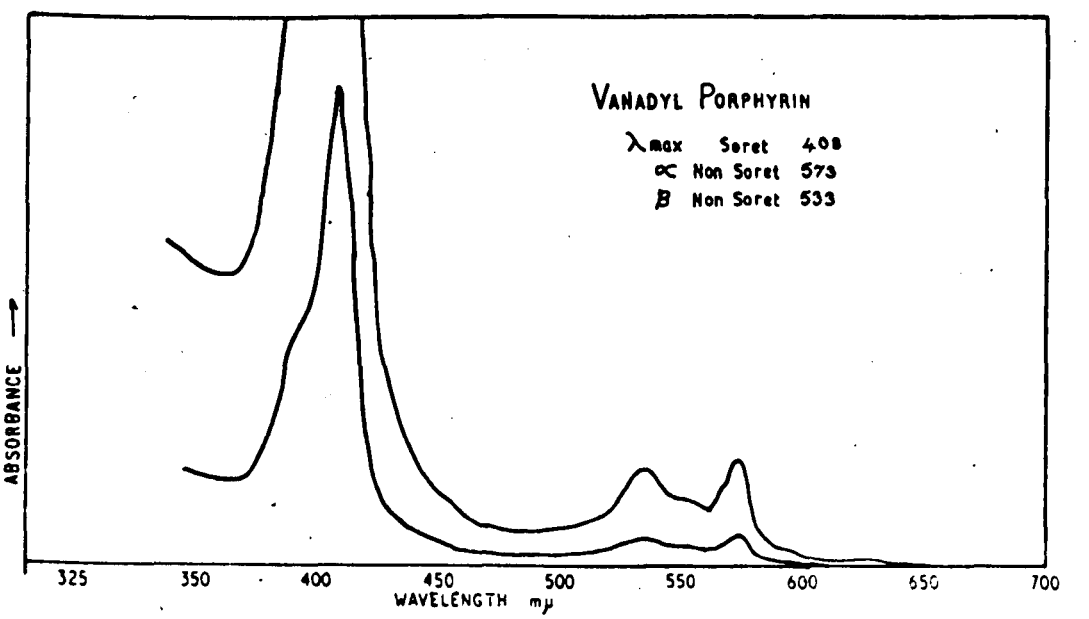
It was found essential to use all three methods of purification before mass spectral identification of the porphyrin chelates since otherwise there was considerable interference, from molecular ions of high molecular weight aromatic compounds. The nickel and vanadyl chelates were examined by m.s. to determine (i) the types of porphyrins present, (ii) the presence of a homologous series and (iii) if vanadium or nickel has any preference for chelation with either the DPEP or MEP types of porphyrin.

Visible light analysis of the silica gel chromatographic fractions, Fig. 25, established the type of chelating metals and a concentration of 37.4 p.p.m. of total metalloporphyrin, per unit weight of Marl Slate. The individual concentrations of metalloporphyrin were found to be –

Figure 25


NICKEL PORPHYRIN
λ max Soret 392
∝ Non Soret 553
β Non Soret 515
ABSORBANCE
325 350 400 450 500 550 600 650 700
WAVELENGTH mμ


NICKEL PORPHYRIN
λ max Soret 393
∝ Non Soret 553
β Non Soret 516
UNKNOWN METALLO PORPHYRIN
λ max Soret 407
β Non Soret 574
ABSORBANCE
325 350 400 450 500 550 600 650 700
WAVELENGTH mμ


VANADYL PORPHYRIN
λ max Soret 408
∝ Non Soret 573
β Non Soret 533
ABSORBANCE
325 350 400 450 500 550 600 650 700
WAVELENGTH mμ

(i) Ni(II) DPEP and MEP; 9.0 p.p.m.

(ii) VO DPEP and MEP; 22.7 p.p.m.

(iii) M(unknown metal)-porphyrin; 4.7 p.p.m.

The identity of the unknown metal chelate was not ascertained. From column chromatographic behaviour on silica gel it was neither iron (Fe^{2+}, Fe^{3+}) nor Vanadyl, since it was eluted too early in the elution scheme. An arc spectrogram of the fraction failed to reveal the identity of the metal atom. A mass spectrum of the fraction revealed a fragment ion at m/e 532 and a homologous series of porphyrins differing in the number of methylene groups substituted on the four pyrrole rings of the porphyrin nucleus. The molecular ion of the unknown porphyrin chelate was not observed and thus the identity of the chelating metal remained unknown.

The content of nickel porphyrins accords well with those found by Hodgson et al[132] in fine grained marine clastic rocks. These authors showed that vanadyl porphyrins had a strong tendency to concentrate in marine sediments, ranging up to a maximum concentration of 39 p.p.m. in the samples studied. They noted that when both nickel and vanadyl chelates were present, the content of the nickel chelate was greater than that of the vanadium chelate in about two-thirds of the samples. In sediments in which the concentration of the vanadyl porphyrin was greater than that of the nickel porphyrin the ratio of nickel porphyrin to vanadyl porphyrin varied between 0.004 to 0.77, with most ratios in the range 0.3 to 0.5.

The ratio of nickel to vanadyl porphyrins in the Marl Slate (Downhill) sample was found to be 0.39 and accords well with the data of Hodgson et al[131]. The important factors that emerge from their work is that nickel porphyrins occur in detectable amounts more frequently in marine than non-marine argillaceous and carbonate sediments and that vanadyl porphyrins are more commonly found in marine sediments.

Mass spectrometry has proved a valuable tool in identifying

porphyrins, and is able to determine the number of peripheral or alkyl carbon atoms and confirm the presence or absence of an isocyclic ring[101]. Identification of homologous series of porphyrins has been achieved by low ionising voltage (ca. 12 e.v.) mass spectrometry. Under these conditions spectra are easily interpreted since principally molecular ions are formed although interference, due to molecular ions of non-porphyrin material, is often present.

Mass spectra of the nickel and vanadyl chelates showed a MEP series and a DPEP series which are evident in Figs. 26 and 27. The former shows the nickel DPEP and MEP homologues with a maximum concentration at m/e 532 and 534 respectively; the latter figure shows the two vanadyl homologous series, with a maximum concentration at m/e 541 and 543 respectively. The most intense members of these homologous series represent the porphyrins mesoetioporphyrin and deoxophylloerythroetioporphyrin, which might be diagenetically derived by a number of processes shown in Figs. 28 and 29, from the possible precursors haemin and chlorophyll a.

A series of reactions has been suggested and reviewed by Hodgson et al[113] to illustrate how chlorophyll a may be altered, by diagenesis, to DPEP (Fig. 28). The sequence of reactions involves the following steps.

(i) conversion to pheophytin a under mildly acidic conditions,

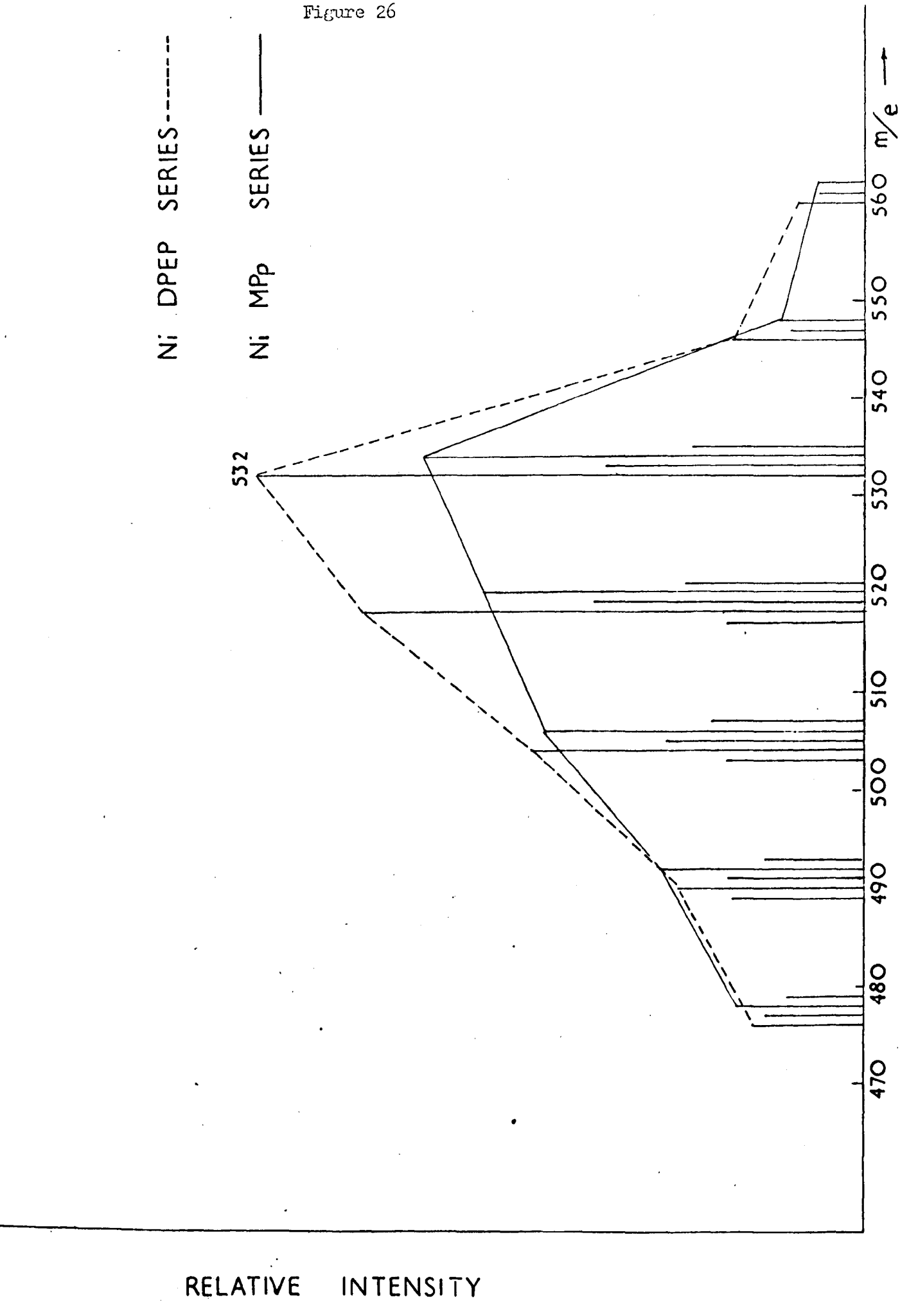
(ii) hydrolysis of the phytyl ester group on the propionic acid side chain of carbon-7 yielding pheophorbide a,

(iii) dehydrogenation at carbon atoms 7 and 8, and hydrogenation of the vinyl group on carbon 2 to yield pheoporphyrin,

(iv) pheoporphyrin spontaneously degrades to phylloerythrin by rapid saponification of the β-ketoester followed by decarboxylation of the resulting free acid,

(v) reduction of the carbonyl group on the isocyclic ring to give deoxophylloerythrin, a carboxylated porphyrin

Figure 26


Ni DPEP SERIES
Ni MPp SERIES
532
560
550
540
530
520
510
500
490
480
470
m/e
RELATIVE INTENSITY

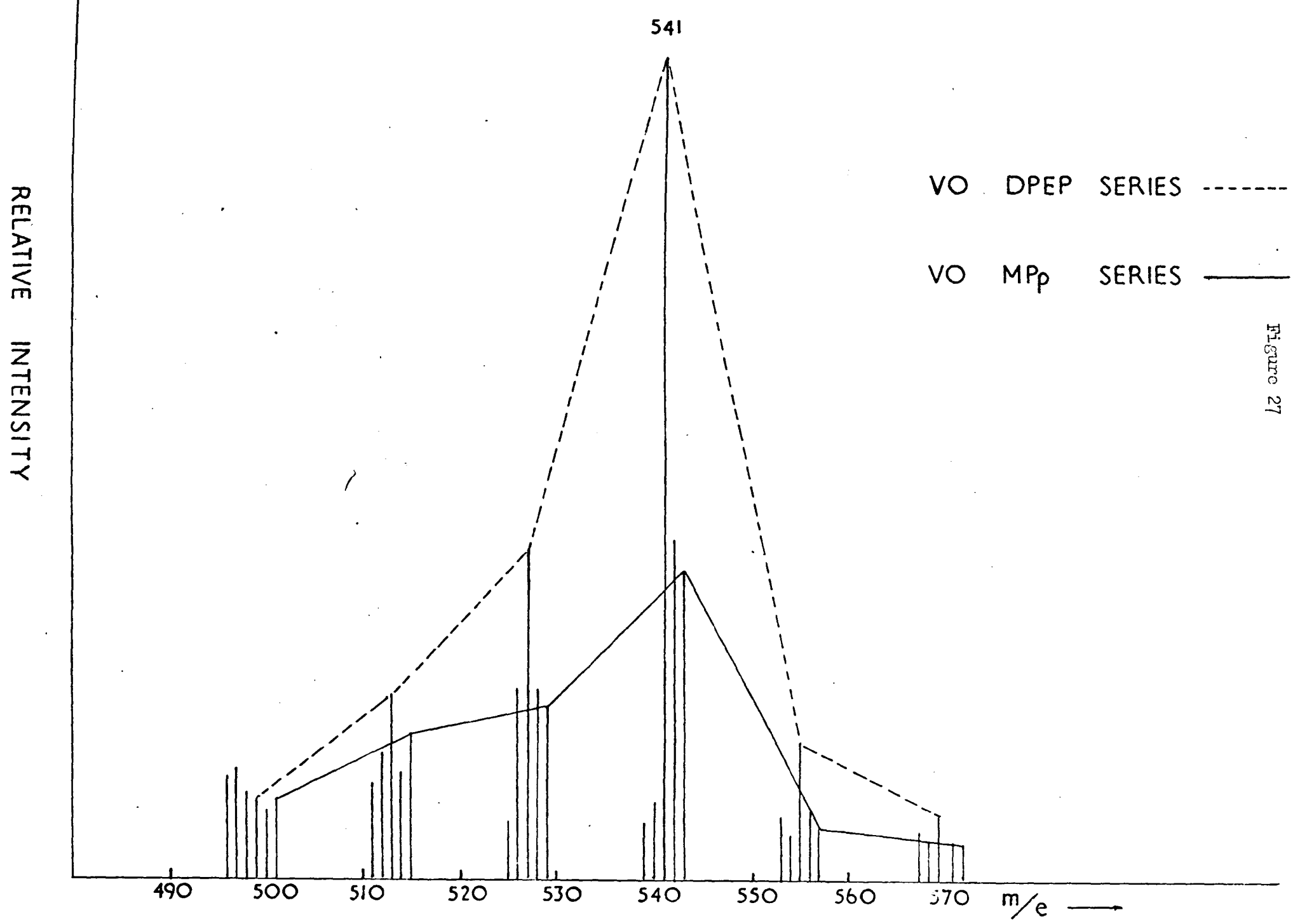
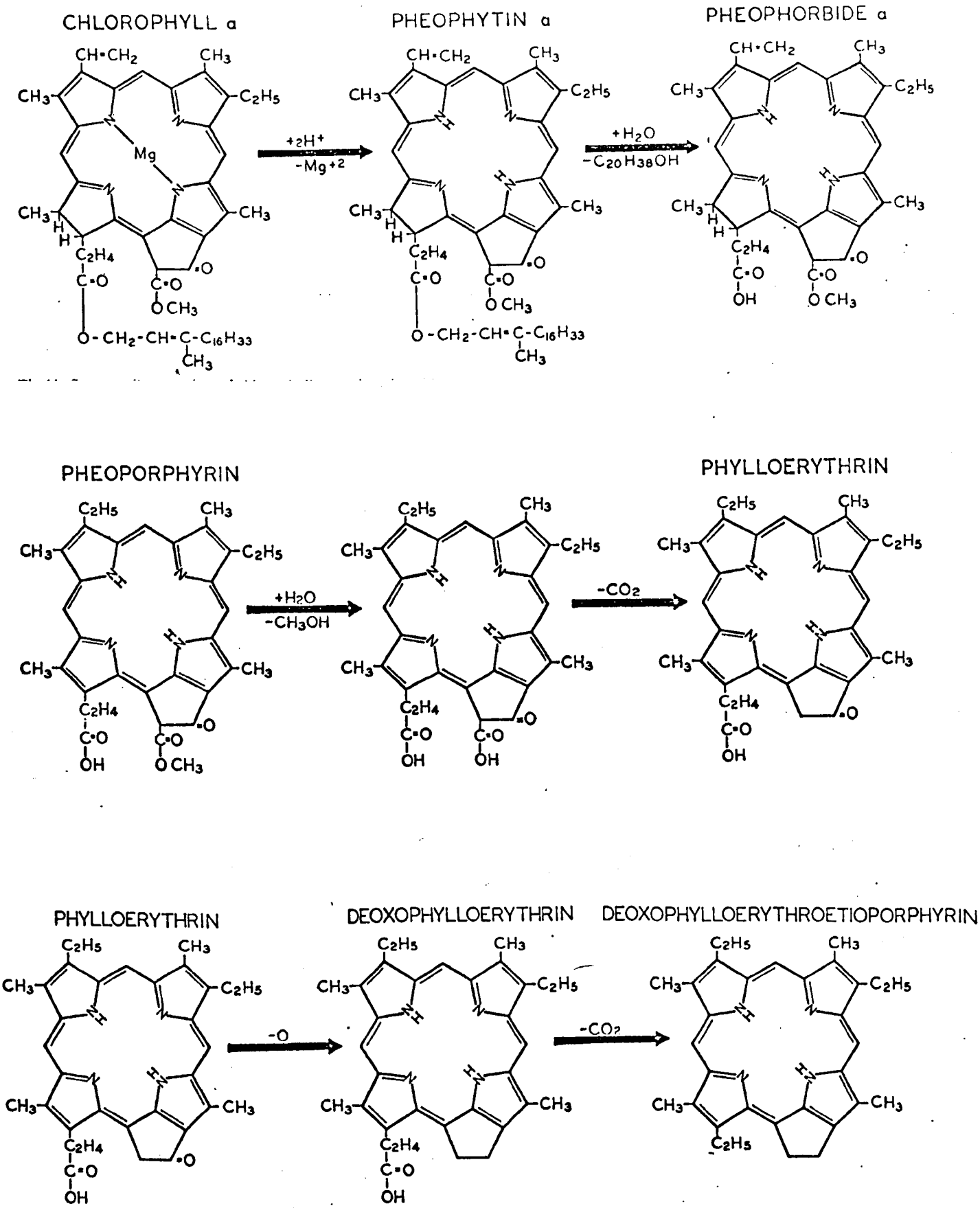

541
VO DPEP SERIES
VO MPp SERIES
RELATIVE INTENSITY
490
500
510
520
530
540
550
560
570
m/e

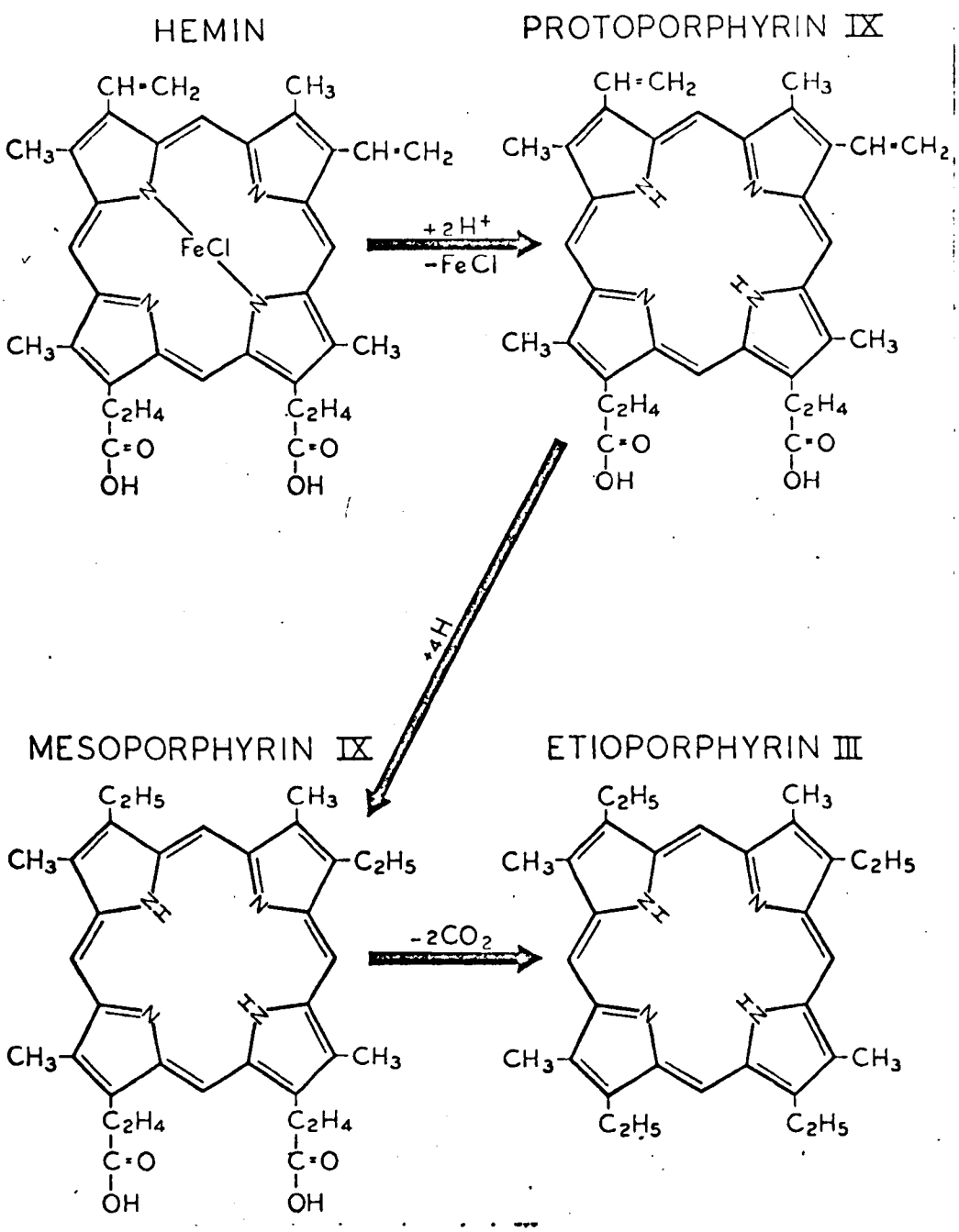
Figure 27

Figure 28



STRUCTURAL CONVERSION OF CHLOROPHYLL a TO DEOXOPHYLLOERYTHROETIOPORPHYRIN

Figure 29



STRUCTURAL CONVERSION OF HEMIN TO ETIOPORPHYRIN III (MESOETIOPORPHYRIN)

occurring in petroleums, and

(vi) decarboxylation of deoxophylloerythrin to DPEP.

This last reaction requires very severe conditions e.g. distillation from alkali at 300°C. Under geological conditions, however, this process would have to occur in many sediments at temperatures below 100°C, thus DPEP has been found in Green River Shale which, it is believed has not been subjected to temperatures greater than about 74°C. Similarly, carboxylated porphyrins of the haemin type could be decarboxylated to mesoetioporphyrin (etioporphyrin III) as shown in Fig. 29.

This sequence of reactions does not however explain the formation of homologous series based on these two porphyrin types, and Baker has suggested that transalkylations of the porphyrin nucleus may occur, as discussed previously. The recent work of Hoering[105] has shown that both DPEP and MEP porphyrins can be formed by heating chlorophyll a in the sediment: However these preliminary findings have yet to be confirmed.

In the Green River Shale the ratio DPEP/MEP = 5.0. This ratio, according to Baker et al[102], suggests a non-marine origin for the porphyrins and it is apparent that little animal derived porphyrins are present since mesoetioporphyrins account for only 20%. The ratios of the porphyrins from the Rozel Point and Santiago petroleums, believed to be of non-marine origin, were found to be 5.8 and 2.9 respectively. These sedimentary environments are geologically very young and have not been subjected, as yet, to high temperatures. The high content of DPEP suggests derivation from chlorophyll a and it is apparent that MEP has not yet been derived from chlorophyll a in the manner suggested by Hoering.

The Marl Slate is substantially older (ca. 270×10^6 yr) and may have been subjected to higher temperatures than the Green River Shale. The ratio of all nickel DPEP/MEP in the Marl Slate is 1.21 and the ratio of all vanadyl DPEP/MEP is 2.07. These ratios suggest

that (i) vanadyl shows a preference to chelate with DPEP or that (ii) DPEP is more stable with respect to degradation of the isocyclic ring when chelated with vanadium than with nickel. Rosscup and Bowman[134] have studied the thermal stabilities of nickel and vanadyl porphyrins at elevated temperatures (373°C, 395°C). They measured the rate constants for the 1st order reaction at these temperatures and calculated the activation energies for demetallation of the porphyrin nucleus to be

43Kcals/mole for vanadyl porphyrin and

46Kcals/mole for nickel porphyrin

These results show that the vanadyl porphyrin is thermally more labile which is in marked contrast to their stability in acidic media[135]. The same results may be inferred for the DPEP and MEP types of porphyrin, chelated with vanadium and nickel, so that it appears reasonable to suggest, from the above data, that the isocyclic ring is more stable to degradation when chelated with nickel than with vanadium. This would invalidate the above suggestion (ii) and imply that kinetic factors control the vanadyl DPEP/nickel DPEP ratio.

These above ratios, although agreeing with those found by Baker et al[102] for marine derived porphyrins, suggest a marine origin for the Marl Slate sediments.

Analysis of the Marl Slate porphyrins has shown that they are qualitatively similar to those found in other sediments. Quantitatively, the Marl Slate is much richer in porphyrins than most ancient sediments so far analysed. This may be due to the unusually high concentration of heavy metals in this sediment, which has allowed chelation with vanadium and nickel which are known to enhance the stability of the porphyrin nucleus[99].

(c) GAS CHROMATOGRAPHY OF PORPHYRIN DERIVATIVES

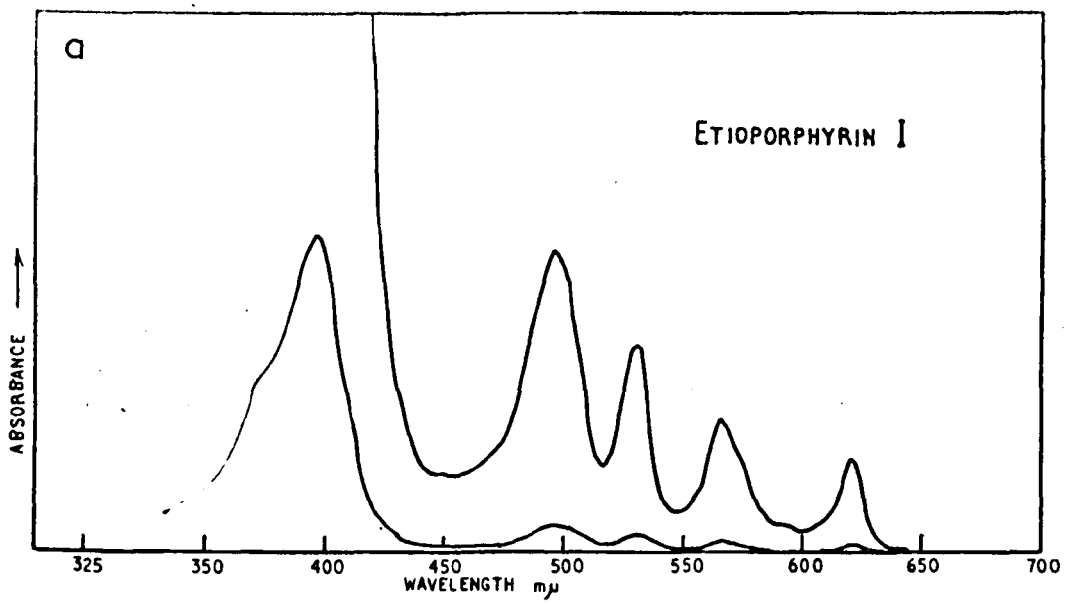
A further aim in the study of the Marl Slate metalloporphyrins was to prepare volatile silicon derivatives of the demetallated porphyrins

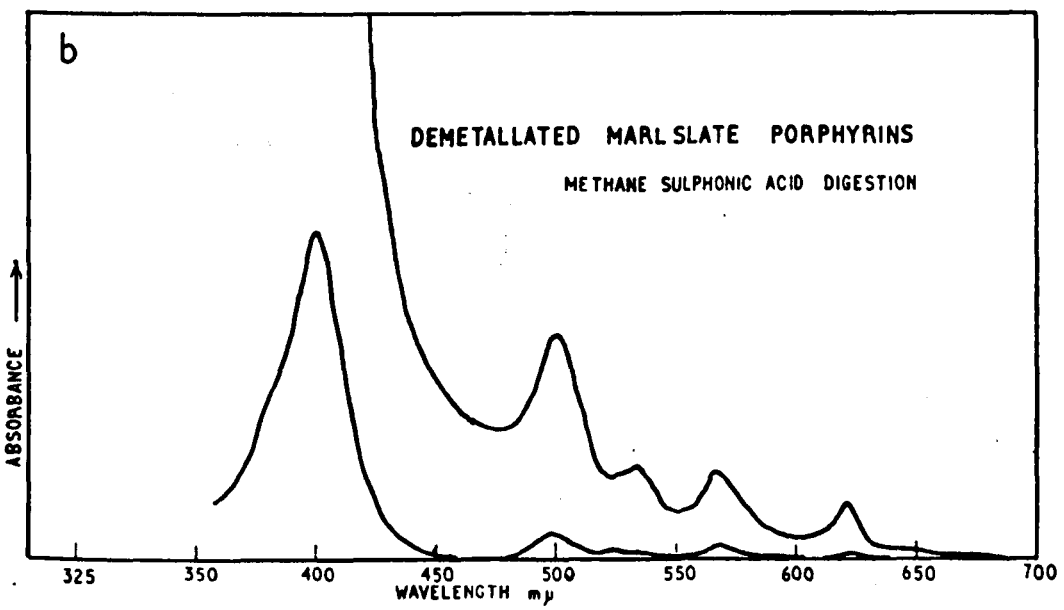
and to attempt g.c. separation of these derivatives according to the method of Boylan and Calvin[119]. A suitable porphyrin was required in large amounts so that the synthesis of these derivatives could be standardised and the method then used for preparing similar derivatives of the Marl Slate porphyrins. Etioporphyrin I was synthesised and purified by chromatography on alumina according to a method kindly provided by Professor A.W. Johnson (unpublished). The visible spectrum of the synthetic product had a Soret band at λ max 395 mμ and non-Soret bands at 496, 532, 566 and 620 mμ, this agreed with a previously published etio-porphyrin spectrum[113]. The mass spectrum of the synthesised standard had a molecular ion at m/e 478 corresponding to the molecular weight of etioporphyrin I.

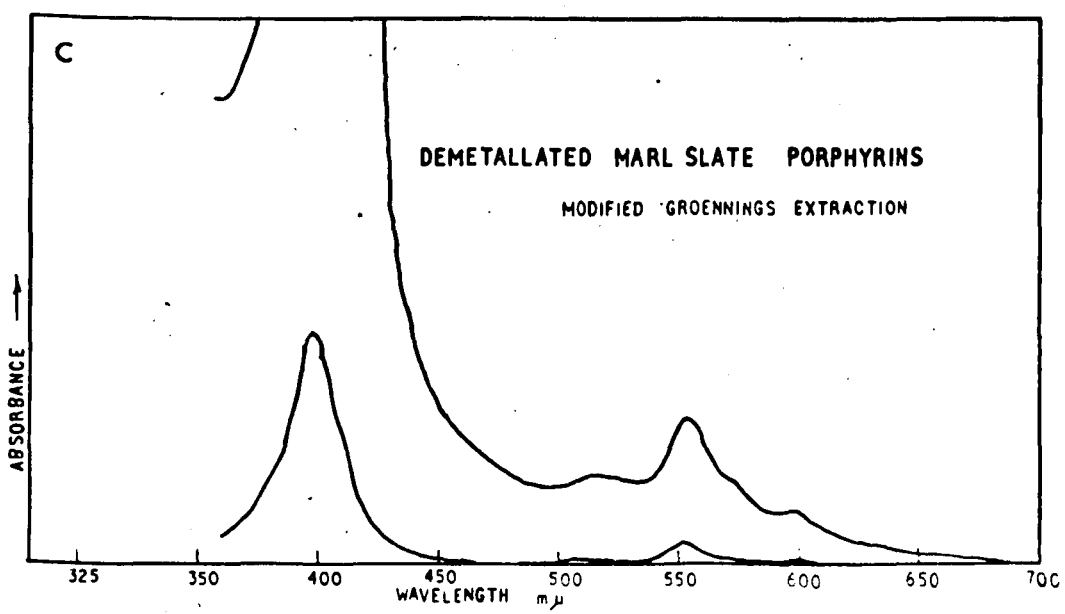
In order to synthesise Marl Slate porphyrin derivatives it was first necessary to demetallate the vanadium and nickel chelated porphyrins. Two methods of demetallation were studied for quantitative yields and ease of experimental procedure. The Groennings method[136] was compared with the more recent procedure patented by Erdman[117]. The latter method was found to be much quicker than the former, requiring only 1 hour, compared to 4-5 days. Both methods were reasonably quantitative (ca. 90%) and yields for the Erdman procedure could be slightly increased by increasing the period of digestion. Visible light spectrophotometry showed that the Erdman method gave a cleaner product (Fig. 30b), and a mixture of etio and phyllo type porphyrins with absorption bands at λ max 399, 500, 534, 567 and 623 mμ were evidently present. This Erdman method was employed subsequently since it was quicker and required less manipulative treatment.

Boylan and Calvin's procedure of preparing dihydroxy-Si^{IV}-etioporphyrin was followed and it was found that the procedure could be modified in order to increase the yield and make handling less dangerous. These authors prepared the dihydroxy-silicon derivative by reacting etioporphyrin I with silicon tetrachloride in pyridine at 180°C for

Figure 30


a
ETIOPORPHYRIN I
ABSORBANCE
325
350
400
450
500
550
600
650
700
WAVELENGTH mμ


b
DEMETALLATED MARL SLATE PORPHYRINS
METHANE SULPHONIC ACID DIGESTION
ABSORBANCE
325
350
400
450
500
550
600
650
700
WAVELENGTH mμ


c
DEMETALLATED MARL SLATE PORPHYRINS
MODIFIED GROENNINGS EXTRACTION
ABSORBANCE
325
350
400
450
500
550
600
650
700
WAVELENGTH mμ

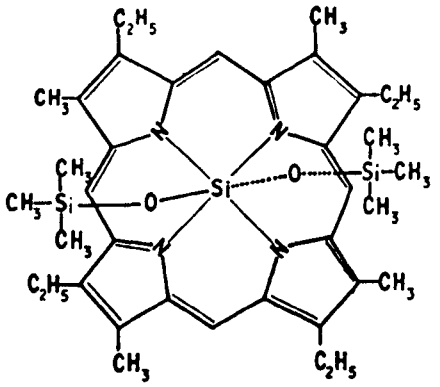
6 hours. Silicon tetrachloride reacts violently with pyridine to form a solid ($SiCl_4$:2Py) octahedral complex[137]. The vapour pressure of this complex has been studied[138] at various temperatures and it is necessary for the complex to be dissociated before incorporation of the silicon tetrachloride moiety can take place in the porphyrin ring. Upon chelation with the porphyrin, the "lone pair" of electrons on the nitrogen atoms of rings II and IV donate their electrons to empty d orbitals of the silicon atom; forming two $d\pi - p\pi$ bonds simultaneous with the removal of two hydrogen chloride molecules from the complex. Pyridine serves as an acceptor by removing the hydrogen chloride from the reaction and preventing acid demetallation of the complex. It was decided to investigate other acceptor molecules which did not react so vigorously with silicon tetrachloride but which would allow the reaction to proceed more quickly, and if possible, produce a higher yield of the dihydroxy-Si^{IV}-etioporphyrin I. The reactions of silicon tetrachloride with amines and amides has been investigated[139,140,141] and trimethylamine was selected for this work because of its high volatility and its low reactivity with silicon tetrachloride with which it forms a 1:1 adduct at $-78^{o}C$. It was found that the reaction was more reproducible and gave slightly greater yields with trimethylamine than with pyridine, these yields being 70% and 60% respectively. The main advantage of using trimethylamine was that it did not react explosively with silicon tetrachloride, otherwise the reaction was carried out in a manner analogous to that of Boylan and Calvin.

The ultraviolet spectrum of the dihydroxy derivative (Fig. 31) was in accordance with Boylan and Calvin's data; the m.s. showed the expected molecular ion at m/e 538.

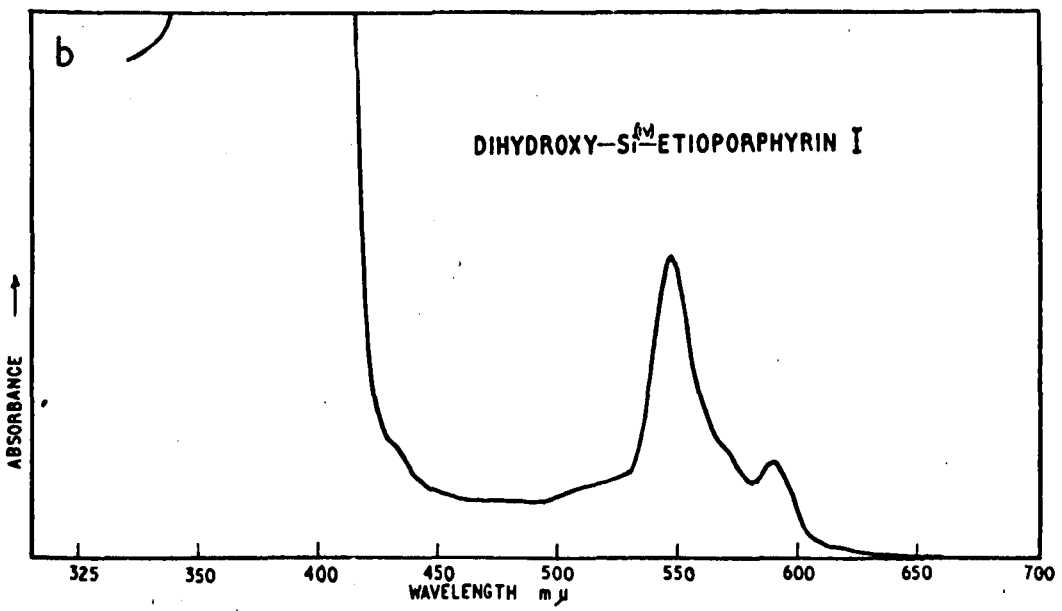
The dihydroxy silicon derivative of etioporphyrin I was silylated with bis(trimethylsilyl) acetamide and its structure is shown in Fig. 31a. (Trimethylchlorosilane and silyl-8 (Applied Science Inc.) were found to give negative results).

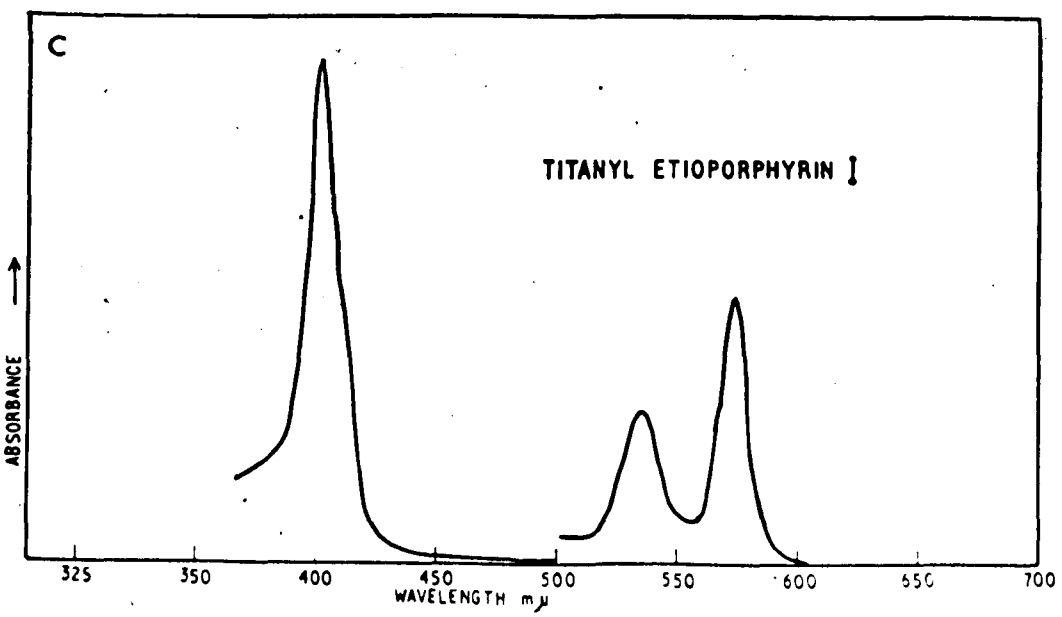
Figure 31

a



BIS (TRIMETHYLSILOXY)–Si$^{(IV)}$–ETIOPORPHYRIN I





The products of several silylation reactions were isolated and purified by preparative thin layer chromatography using benzene/10% chloroform as developer. Mass spectra of the recovered derivatives were recorded at 70 e.v. with a source temperature ranging from 150°C to 250°C. The spectra were fairly simple at the high molecular weight end, showing a molecular ion at m/e 682 and a fragment peak at m/e 593 due to loss of the siloxy group ($O-Si(CH_3)_3$).

It was evident from thin layer chromatography and mass spectrometry that the yield of the di-silylated compound was poor (8%). Byproducts of the reaction were found to be the monosilylated compound (32%) with a molecular ion at m/e 610, and a silylated porphyrin derivative showing a molecular ion at m/e 612. This latter molecular ion may have been produced in the mass spectrometer by the silicon atom assuming a tetra-coordinate structure and the two pyrrolic nitrogens abstracting hydrogen atoms from another porphyrin molecule. It was not possible to increase the yield of the disilylated product, bis(trimethylsiloxy)-Si^{IV}-etioporphyrin I, and this was a serious drawback in the attempted g.c. separation of the Marl Slate porphyrins.

At this time, Boylan succeeded in synthesising the disilylated derivatives of porphyrins isolated from Boscan crude oil. (Prior to this the preliminary report[119] contained few experimental details). The synthesis of the disilylated porphyrin Si^{IV} compounds has been found to proceed with difficulty although Boylan* has managed to produce them in greater yield. The R_f values of the various Si^{IV} derivatives of etioporphyrin I and Marl Slate porphyrins are reported in this thesis (cf. experimental section) and provide a rapid check of the course of the reactions involved.

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* Personal communication.

G.c. separations of the disilylated derivatives of etioporphyrin I and Marl Slate porphyrins were attempted on several columns (cf. experimental section). Partial success was obtained only on low resolution 4ft x $\frac{1}{8}$" i.d. glass columns packed with silanised Corning glass beads coated with OV-1 (a silicone gum). It was observed that considerable adsorption of the disilylated derivative occurred and only by repeated injections was it possible to elute the porphyrins. Ultra-violet inspection of the glass beads showed that the Si^{IV} -porphyrins had not in fact undergone demetallation. Demetallated porphyrins do not fluoresce in the solid phase.

Although the final aim of this part of the thesis has not been successful it may be mentioned that Boylan has managed to obtain g.c. separations which, although not very informative, shows that g.c. separation, however poor, can be achieved. Similar attempts, by Hodgson, have also proved unsuccessful.

Attempts were made to form a hexa-coordinate porphyrin derivative with titanyl tetrachloride but mass spectrometry of the hydrolysed derivative indicated a molecular ion at m/e 540 corresponding to the presence of etioporphyrin I chelated with the titanyl group (Ti = O), the visible spectrum of this compound is shown in Fig. 31c. The structure is probably similar to that of the vanadyl porphyrin[115]. Titanium does form hexa-coordinate structures but the dihydroxy derivative is apparently unstable eliminating water and forming the square pyramidal structure noted here.

At the present time, high pressure gas liquid chromatography of porphyrins is not perfected and it is not possible to analyse carboxylated porphyrins due to their relative insolubility in the liquid carrier gases used.

Germanium derivatives of porphyrins may prove of interest in the search for other metals which may form hexa-coordinate chelated structures

with porphyrins but a major difficulty may be in increasing the molecular weight of the porphyrin derivatives and thus causing reduced volatility.

Mass spectrometry, though a valuable tool in the identification of porphyrins and their homologues, is restricted in that it cannot determine the positions of substitution of the peripheral alkyl groups on the porphyrin nucleus. T.l.c. does not provide sufficient resolution for separating porphyrin homologues at the present time. It may prove possible to separate and analyse porphyrins by combined g.c. m.s. If the porphyrin derivatives could be separated by high resolution capillary chromatography it might prove possible, by synthesis of the isomers of these individual porphyrin homologues, to determine whether isomerisation has occurred during diagenesis within the sedimentary environment. G.l.c. appears to be the only technique available by which structural isomers of porphyrins may be separated. Without this technique it may prove difficult to understand the diagenetic changes which have been responsible for reducing the naturally occurring porphyrin types to those present in the geological environment.

CHAPTER VI

KEROGEN IN THE MARL SLATE

(a) INTRODUCTION

The amount of soluble organic matter in sediments generally represents a small fraction only (ca. 1%) of the total organic carbon content of the rock. Recently interest has been directed towards the insoluble organic material which is referred to as kerogen. This interest has been twofold; firstly to understand the chemical nature, structure and structural differences of various kerogens and secondly to determine whether kerogen represents an intermediate stage in the genesis of petroleum. An adequate definition of kerogen is not possible at the present time since the chemical complexity of this organic material is difficult to characterise. Kerogens isolated from different sedimentary sources show marked elemental and physical differences and only a generalised definition is possible. The author suggests that the best definition that can be presented is that kerogen is a heterogeneous chemically complex polymer of variable molecular weight, formed by the self-condensation of organic material accumulating in the sedimentary environment, which may undergo subsequent chemical changes during its period of incorporation within the sediment. Only a generalised definition is possible since in a typical marine sediment there is a great variety of organic debris which ultimately, under the effect of diagenesis, is transformed into a kerogen. Thus, on the basis of microscopical studies, algal, planktonic, mollusc and bacterial debris etc. constitute important precursor constituents of kerogens[redacted]. Also it is important to note that a particular kerogen does not maintain a constant elemental and structural composition over prolonged periods of time[142]. Due to the effects of thermal metamorphism a gradual decrease in hydrogen, oxygen and nitrogen occurs with a concomitant increase of aromatic structures. The effects of time, temperature and depth of burial on the structural composition of coals and lignites has been studied by Karweil[144].

Methods for the separation of kerogens from the inorganic constituents

of sediments have been previously reviewed. The general physical and chemical properties of kerogens has been reported by Forsman[145]. From data available prior to 1963, Forsman has classified kerogen into at least three types, namely,

(i) coaly type - consisting predominantly of condensed aromatic structures,

(ii) aliphatic type - containing cycoalkane, mononuclear aromatic and open chain structures,

(iii) coal-oil shale type-possessing structures intermediate between the above.

Subsequent work by Robinson and Dineen[143] on twelve different oil shales has revealed major differences in the structure of their associated kerogens. The kerogens were analysed for elemental composition and were subjected to oxidation, pyrolysis, functional group and i.r. analyses. The differences in the constitution of the kerogens were related to the age, depositional environment and type of source material of the oil shale. I.r. spectra of these kerogens revealed that young kerogens contain greater amounts of carboxyl, ester and hydroxyl groups than older kerogens. The transformation of kerogen in a Triassic (ca 200×10^6 yr) shale formation has been studied by Long et al[146]. The formation has undergone a differential heating due to metamorphic events in its past history. Transformation of the kerogen has occurred, probably by a disproportionation process, which has led to progressive cyclisation and aromatisation of the kerogen and the formation of gaseous products, chiefly methane. Studies involving oxidation[147-150], pyrolysis[151-153], saponification[76], and hydrogenolysis[154] have been important in providing a knowledge of the chemical structure of kerogen. Some of these studies have also shown the similarity of degradation products to some constituents of petroleum. High pressure hydrogenolysis, as a means of degrading polymeric substances, has been one of the most effective methods in the structural study of coal[155], and soils[156]. Hubbard and Fester[154] extended this technique to a study of the Colorado oil

shale kerogen. By utilising a stannous chloride catalyst at 355°C under a H_2 pressure of 4,200 psi they were able to solubilise 82-88% of the kerogen. From the benzene soluble fraction they were able to isolate long chain waxes (12%), and branched cyclic (26%), hydrocarbons and nitrogen and oxygen containing heterocycle compounds (36%). Unfortunately, only the physical properties of these fractions were recorded. With the development of improved chromatographic techniques, an analysis of the constituents of this hydrogenolysis product becomes possible. The separation of morphologically preserved fossil organic components i.e. spores from coals of Carboniferous age (ca 320 x 10^6 yr) and the determination of structural components within this type of kerogen has been reported[157]. The development of other degradative techniques and their systematic use on various kerogen types should prove important in elucidating structural components and thus help to formulate a more knowledgeable assessment towards the nature of kerogen.

(b) HYDROGENOLYSIS OF MARL SLATE KEROGEN

Hydrogenolysis studies on Marl Slate kerogen concentrate were carried out using essentially the same experimental conditions that were used by Hubbard and Fester[154]. These authors studied the most effective catalysts required for maximum conversion of Colorado oil shale kerogen to benzene soluble material with a minimum production of gas. They subsequently found that these conditions were best satisfied by heating 2.6 parts of kerogen concentrate and 1 part of stannous chloride dihydrate with hydrogen at 355°C and 4,200 p.s.i. (hot pressure) for four hours. They noted that temperature was the most important factor in increasing the gas (chiefly methane) yield and that at 375°C the gas yield was increased threefold.

The aim of the following study was twofold. Firstly, to degrade the Marl Slate kerogen to benzene-soluble material in order to determine the amount of long chain aliphatic hydrocarbons present in the kerogen.

TABLE 9. RESULTS OF HYDROGENOLYSIS OF MARL SLATE KEROGEN CONCENTRATE (C42%, H4.5%)*

	Benzene-soluble material (g)	%C,H	n-alkanes (mg)	branched/cyclic alkanes (mg)	total alkanes (mg)
320°C (2hrs) 20g.	8.20	C,72.5;H8.6%	85	712	1090
280°C (2hrs) 20g.	2.90	C,73.2;H7.29%	25	130	186
200°C (64hrs) 20g.	2.84	n.d.	12.4	81.2	118
175°C (115 hrs) 17g.	0.76	n.d.	5.5	36.1 (alkenes 9.0)	60

* Kerogen concentrate was not treated to remove pyrite.

This required initial experiments to be carried out in order to determine the optimum conditions necessary for maximum solubilisation of the kerogen.

Secondly, if kerogen could be degraded at lower temperatures, larger units might be cleaved and hydrogenated. Structural elucidation of these fragments would help in evaluating the possible building blocks of the kerogen. It was also considered pertinent to determine if the type and distribution of saturated hydrocarbons produced were altered by hydrogenolysis experiments at different temperatures.

Hydrogenolysis studies were carried out at temperatures of 320°C, 280°C, 200°C, and 175°C for varying periods of time. It was necessary to extend reaction times at lower temperatures so that sufficient saturated hydrocarbons could be recovered from the benzene soluble material and separated into normal and branched/cyclic fractions by means of 5A molecular sieve. Results of the hydrogenolysis experiments are reported in table 9.

It is evident from the carbon and hydrogen analyses that the kerogen and the benzene soluble products of the 320°C and 280°C experiments are substantially aromatic. If the results are normalised to a C_6 carbon unit then the kerogen has an elemental formula, $C_6H_{7.7}$, and the 320°C and 280°C benzene soluble products, $C_6H_{8.5}$ and $C_6H_{7.2}$, respectively. It is apparent that little hydrogenation of the benzene soluble products has occurred, indeed, the latter figure suggests that some of the soluble material may have acted as a hydrogen donor in this reaction.

From the results of the 320°C hydrogenolysis it is apparent that approximately 67% of the organic carbon in the kerogen has been solubilised. This shows that the fundamental aromatic building units of this kerogen are of quite low molecular weight since the predominant reaction is cleavage between heteroatoms and not hydrogenation or carbon-carbon bond cleavage. (If condensed aromatic hydrocarbons larger than chrysene ($C_{18}H_{12}$) were present they would neither dissolve easily in benzene nor

would they be hydrogenated under the conditions used.

It is also clear from Table 9, that approximately 12% of the kerogen carbon and hydrogen is in the form of aliphatic hydrocarbons chemically bound to the more aromatic building units of the kerogen. The amount of saturated hydrocarbons liberated from the Marl Slate (Downhill) sample, assuming that at least 12% is present as long chain aliphatic hydrocarbons, is more than 15 times that which can be extracted from the shale. It is thus apparent that even when the kerogens of various geological samples are substantially aromatic in character, a considerable amount of long chain hydrocarbons can still be produced from them.

The alkanes in the hydrogenolysis products from the experiments at geologically more viable temperatures (i.e. 175°C) show that kerogen, even though aromatic in character, can be regarded as a precursor of "petroleum like" hydrocarbons. It is not implied that the kerogen is chemically homogeneous since fluorescence microscopy has shown that the organic material is heterogeneous in character and contains spores, hystrichospheres, etc. The above generalisations are based on the overall composition of the kerogen. It is known from coal chemistry[155] that certain coal macerals, e.g. exinite (believed to be derived from spores, cuticles, algae and resins) and vitrinite (lignin derived), lose their morphological identity at certain stages of coalification. The rank of the coal is dependent essentially upon the temperature to which the coal has been raised during burial. The effects of time, temperature and pressure on the coalification process has been discussed by Karweil[144]. Marl Slate hydrogenolysis experiments were carried out at different temperatures to determine qualitative differences in the types of aliphatic hydrocarbons produced. Although the precursor organic material from which the kerogen was derived was not totally of plant origin it was considered that hydrogenolysis at a particular temperature would favour the cleavage of the weakest heteroatomic bonds, the nature

of crosslinking being dependent on the particular cellular constituents of the macerals.

The total, branched/cyclic, and normal alkanes obtained from the benzene-soluble products were analysed by g.c. so that it was possible to observe qualitatively and quantitatively any differences in the types of alkanes, and their distributions produced at the different temperatures employed.

The gas chromatograms of the total, branched/cyclic and normal alkanes shown in Figs. 32-35 were obtained for each of the hydrogenolysis products and they show several similar features.

Firstly, a homologous series of normal alkanes are present ranging from about from $\underline{n}C_{11}$ to $\underline{n}C_{30}$, with maxima at C_{18} and C_{20} (except for the 320°C hydrogenolysis experiment where the normal alkanes have a maximum at C_{15} and C_{17}).

Secondly the gas chromatograms of the branched-cyclic alkanes show a complex mixture with a number of peaks, three of which have been tentatively identified as phytane, pristane, and the C_{18} isoprenoid hydrocarbon by comparing their retention indices with those recorded for the Marl Slate isoprenoids isolated in extraction experiments.

Thirdly it is evident that high molecular weight branched/cyclic alkanes occur in these products since the gas chromatograms, Figs. 32-35, show a complex unresolved pattern above nC_{26}.

The results of the 320°C hydrogenolysis show that a smooth envelope of normal alkanes is produced with a CPI index of almost unity (Fig. 32). The alkane envelope has a maximum at $\underline{n}C_{15}$ and $\underline{n}C_{17}$ and the distribution of the homologous series gradually decreases until at $\underline{n}C_{33}$ the contribution is less than 1%. Losses of normal alkanes below about $\underline{n}C_{15}$ due to the experimental procedure has been noted before. The branched/cyclic chromatogram though complex shows peaks with the same retention indices as phytane, pristane, and the C_{18} isoprenoid hydrocarbon. It is quite probable that the C_{16}, C_{15} and C_{14}

Fig. 32

Saturated hydrocarbons extracted from the hydrogenolysis products (320°C) of the Marl Slate (Downhill) kerogen

A. Normal alkanes

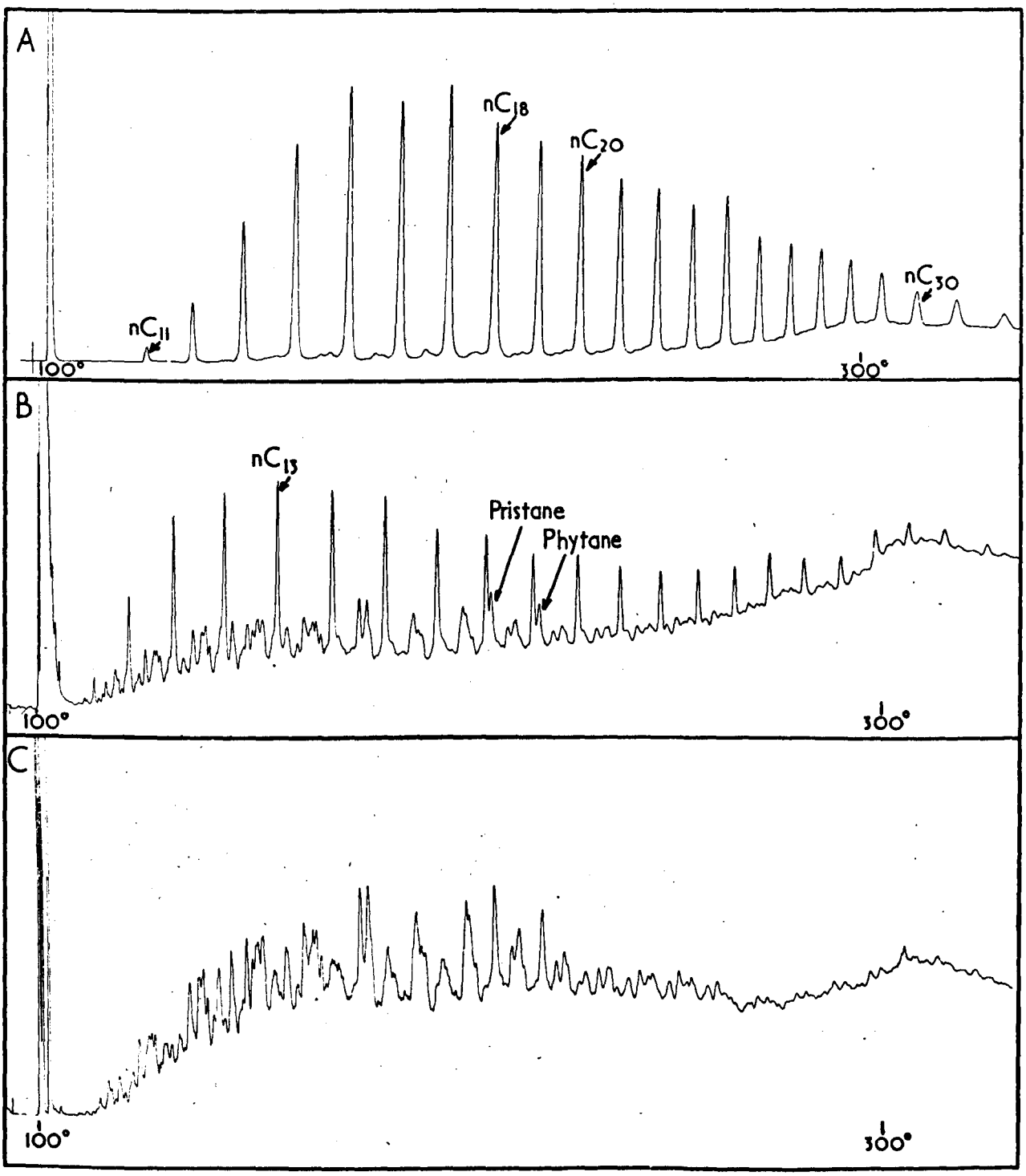
Chromatographic conditions: column 20 feet x 0.080", packed with 3% OV-1 on Gas Chrom Q (100/120 mesh). Oven programmed at 2°C/min between 100°-300°C. Nitrogen carrier gas 18 ml/min. Injector temperature, 300°C; detector temperature 310°C.

B. Total alkanes

Chromatographic conditions: as above.

C. Branched-cyclic alkanes

Chromatographic conditions: as above.


A
nC18
nC20
nC30
nC11
100°
300°
B
nC13
Pristane
Phytane
100°
300°
C
100°
300°

isoprenoid alkanes are present though there are a great number of unresolved peaks and interpretation in this region from retention data only is difficult.

Results of the 280°C hydrogenolysis, Fig. 33 show a qualitative difference from the above. In the distribution of normal alkanes which range from C_{10} to C_{28}, it is apparent that there is a preponderance of the $\underline{n}C_{18}$ and $\underline{n}C_{20}$ alkanes. The branched/cyclic alkanes from this experiment are very complex and appear similar to those of the 320°C experiment. These even membered $\underline{n}C_{18}$ and $\underline{n}C_{20}$ alkanes are even more prominent in the 200°C hydrogenolysis experiment and it is apparent that a series of normal alkanes has been produced with a CPI index markedly less than unity.

Kerogen hydrogenolyses should favour equally the production of all alkanes that are chemically bound to the kerogen with the same type of heteroatom crosslinking. Jurg and Eisma[34] have shown that decarboxylation of an even membered fatty acid in the presence of clay produces predominantly the expected odd-numbered hydrocarbon. The even member hydrocarbons produced in these hydrogenolysis experiments could not therefore arise by decarboxylation of a parent even-numbered acid. It is possible that these alkanes may have been derived from normal even numbered carbon chains which have been attached to the kerogen possibly by an ether or sulphide linkage.

Cleavage of the $\underline{n}C_{18}$ and $\underline{n}C_{20}$ alkyl moieties have occurred more readily at lower temperatures. The disappearance of these even membered homologues at higher temperatures is possibly due to dilution caused by the greater yield of other normal alkanes, the effect of which is to produce a smoother envelope as can be seen in Fig. 21.

The results of the 175°C hydrogenolysis, Fig. 35, show that the $\underline{n}C_{18}$ and $\underline{n}C_{20}$ are even more prominent. It is interesting to note that in this low temperature hydrogenolysis unsaturated aliphatic hydrocarbons are being produced which together form about 15% by weight

Fig. 33

Saturated hydrocarbons extracted from the hydrogenolysis products (280°C of the Marl Slate (Downhill) kerogen

A. Normal alkanes

Chromatographic conditions: see Fig. 32.

B. Total alkanes

Chromatographic conditions: as above.

C. Branched-cyclic alkanes

Chromatographic conditions: as above.

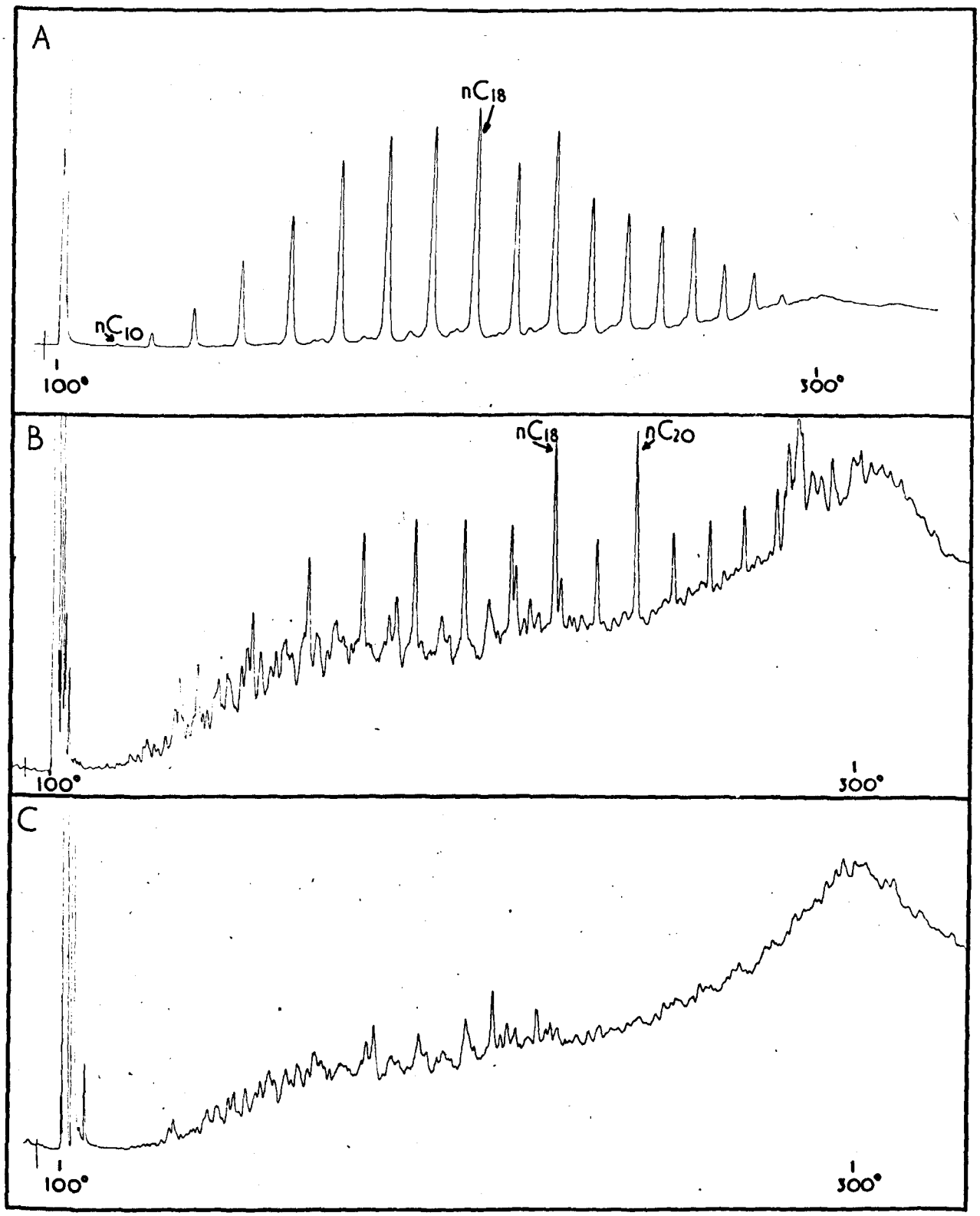

A
nC18
nC10
100°
300°
B
nC18
nC20
100°
300°
C
100°
300°

Fig. 34

Saturated hydrocarbons extracted from the hydrogenolysis products (200°C) of the Marl Slate (Downhill) kerogen

A. Normal alkanes

Chromatographic conditions: see Fig. 32.

B. Total alkanes

Chromatographic conditions: as above.

C. Branched-cyclic alkanes

Chromatographic conditions: as above.

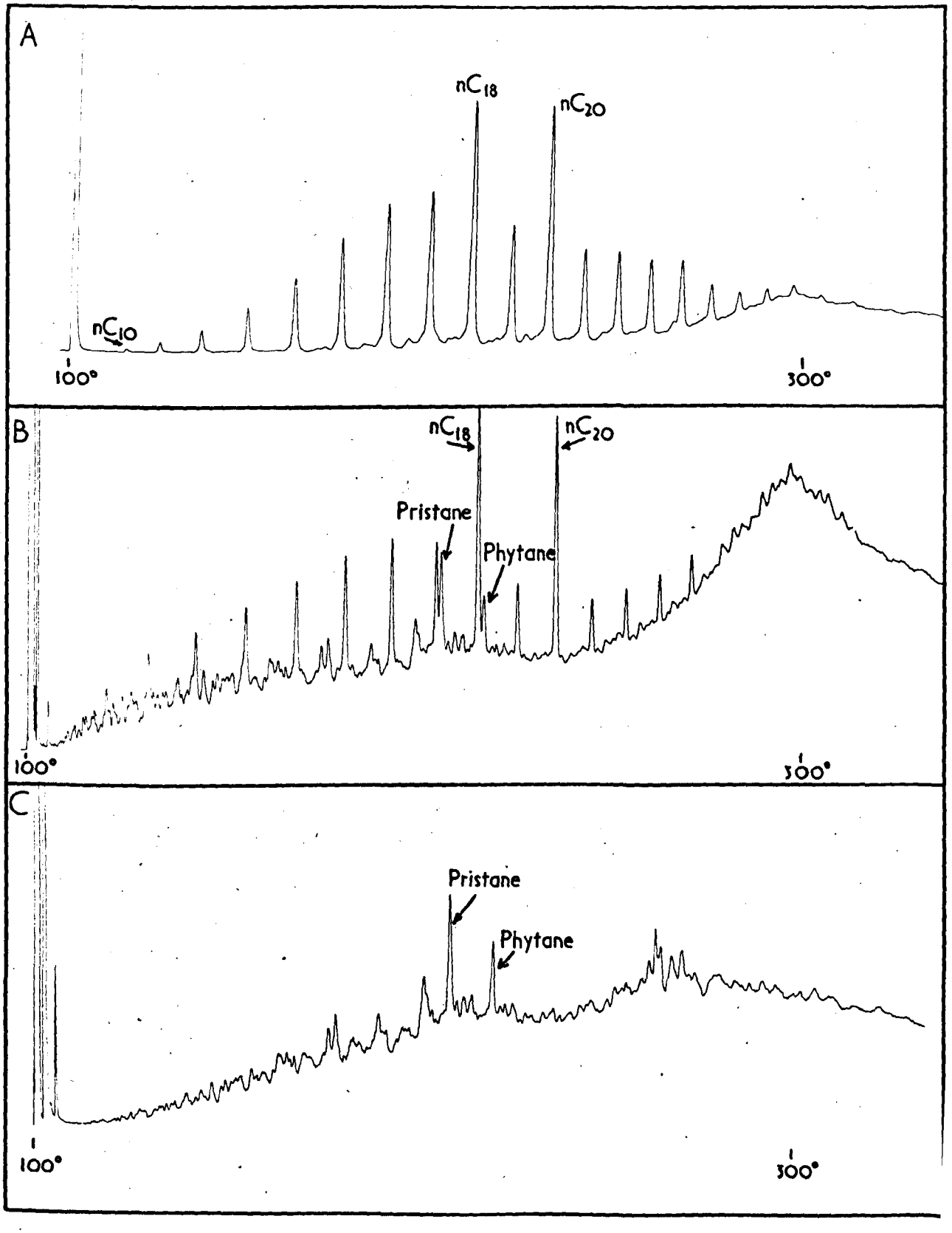

A
nC18
nC20
nC10
100°
300°
B
nC18
nC20
Pristane
Phytane
100°
300°
C
Pristane
Phytane
100°
300°

Fig. 35

Hydrocarbons extracted from the hydrogenolysis products (175°C) of the Marl Slate (Downhill) kerogen

A. Total alkanes

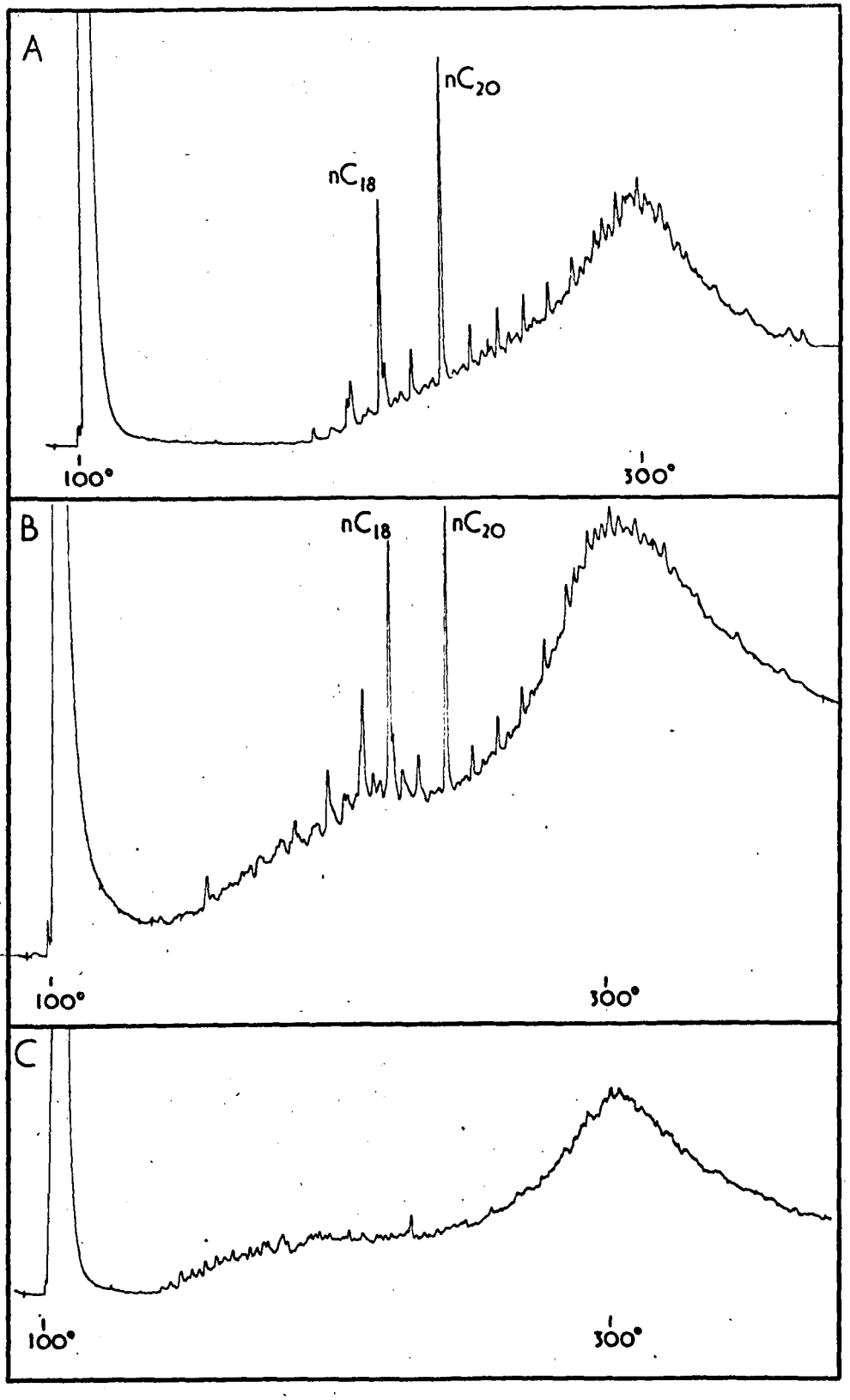
Chromatographic conditions: see Fig. 7.

B. Total alkanes and alkenes

Chromatographic conditions: as above.

C. Total alkenes

Chromatographic conditions: as above.


A
nC20
nC18
100°
300°
B
nC18
nC20
100°
300°
C
100°
300°

of the total alkanes. I.r. analysis showed the presence of long chain aliphatic hydrocarbons containing no aromatic bands. Bands at 812 cm^{-1} and 965 cm^{-1} suggested the presence of tri-substituted and trans-substituted double bonds. The absence of bands at 990 and 910 cm^{-1} indicate that terminal alkenes were not produced. No evidence was obtained for alkenes in the hydrogenolysis experiments conducted at higher temperatures no doubt because olefins were hydrogenated at these higher temperatures.

The gas chromatogram of the alkene fraction, Fig. 35, was complex with no prominent peaks and in this respect, differs from the results of Henderson et al[62] obtained by pyrolysis of Green River Shale.

It is apparent that hydrogenolysis at lower temperatures favours the production of alkenes and these may possibly be hydrogenated by disproportionation or hydrogenation reactions during burial. Results from Northumberland shales of Carboniferous age revealed the presence of what appears to be substantial amounts of alkenes in the solvent extracts of these shales;it is possible that these have been generated by mild thermal treatment of these shales over a long period. Chemical and physical data of oils and shales from the Ventura Basin, California, obtained by Philippi[158],has shown that the bulk of the oil generated has occurred at temperatures in excess of 115°C. Results of the hydrogenolysis experiments show that appreciable amounts of hydrocarbons can be produced at 175°C after only several days of reaction. Thus, temperatures of 115°C or less during prolonged periods of burial may be a sufficient condition for petroleum generation, from kerogens.

The normal alkanes, contained in the soluble extract of Marl Slate extend from C_{12} to C_{32}, with maxima at $\underline{n}C_{17}$ and $\underline{n}C_{19}$. There is no evidence of prominent $\underline{n}C_{18}$ and $\underline{n}C_{20}$ alkanes. This may be due to the fact that the Marl Slate sediments in Co. Durham have had a low temperature history and no contributions from the kerogen has yet occurred.

Preliminary attempts have been made to determine the types of hetero-atomic linkages that are present in the kerogen. The kerogen was subjected to a desulphurisation reaction using a Raney nickel catalyst in methanol and the products were chromatographed on an alumina column. Successive elutions with petroleum ether, benzene, chloroform and methanol failed to yield significant amounts of organic material. In order to determine whether any ether linkages were present in the kerogen, a sample was heated with a concentrated solution of hydrogen bromide in acetic acid, under reflux. The reaction mixture was hydrolysed with 6N potassium hydroxide solution and extracted with chloroform to remove neutral lipids. The chloroform extracts were then chromatographed on alumina. No evidence, i.r. and g.c., for the presence of fatty alcohols was found in any of the eluents. The reaction employed would have caused cleavage of aliphatic ether linkages in the kerogen. It is evident that the aliphatic moieties of the kerogen are not bound to the predominant aromatic structures by aryl or alkyl ether bonds since the reaction employed would have yielded significant amounts of fatty alcohols. It may also be noted here that no evidence was found for the presence of significant amounts of fatty alcohols in the organic solvent extracts of the Marl Slate. These facts and the evidence of very low amounts of fatty acids in the Marl Slate suggests that oxygen groups are very rare in the Marl Slate, in marked contrast to the organic material in the Green River Shale which has been discussed previously.

The results of the hydrogenolysis experiments have shown that about 12% of the Marl Slate kerogen is aliphatic in structure. The distribution of hydrocarbons which constitute this amount is seen in Fig. 32. It is evident that n - alkanes are major contributors to the aliphatic part of this kerogen. The results reported here are the first attempts that have been made to quantitatively determine the

amount, types and distributions of aliphatic organic material in a kerogen. It is thought that more detailed studies may help in elucidating the types of aliphatic - aromatic linkages that are present in this and other kerogens.

PART 2B

THE BOVEY TRACEY LIGNITE

CHAPTER VII

GEOLOGICAL SETTING OF BOVEY TRACEY LIGNITE

The Bovey Tracey lignite deposits, extending between the villages of Bovey Tracey and Newton Abbot in Devon, are found in a basin which originated as a rift valley. Subsidence of this basin below the level of the drainage outlet caused the formation of a lake in which were laid down the deposits, now referred to as the Bovey Beds (Fig. 36). These deposits are considered to be Oligocene in age (ca. 26×10^6 years).

In this lake, measuring about 10 miles by 4 miles, rafts of plant debris and clays accumulated to a depth of over 600 feet. Reid[159] has noted that the depth of the original unconsolidated sediments was substantially greater than the present proven depth, probably by a factor of two.

The Bovey Beds consist of beds of sand and kaolinite clays, derived from the granitic moors of Dartmoor, alternating with lenticular seams of lignite. Throughout the succession, the lignite contains twigs of Sequoia couttsiae which constitute about 99% of the deposit. During times of flood, rafts of plant debris, originating from the Dartmoor area, were swept into this lake. Few aquatic plants and little animal life are believed to have been present in this lake and the presence of swamp vegetation has only been established by the scale of a cone belonging to the swamp cypress Taxodium distichum. No evidence of fish or molluscs inhabiting this former lake have been found[159]. Fragments of fern, seeds of more than one species of Nyssa, a climbing palm, a bramble and some unknown seeds have been found.

The photographs shown in Fig. 37 represent ultra-violet fluorescent leaf structures and resin bodies within a lignite sample. Photographs A and B show cross-sections of large ducts or reservoirs which were originally occupied by solutions or suspensions of resin. These ducts are now completely filled with resins, showing a strong yellow

Figure 36. Geological map of the Bovey Tracey Lignite.

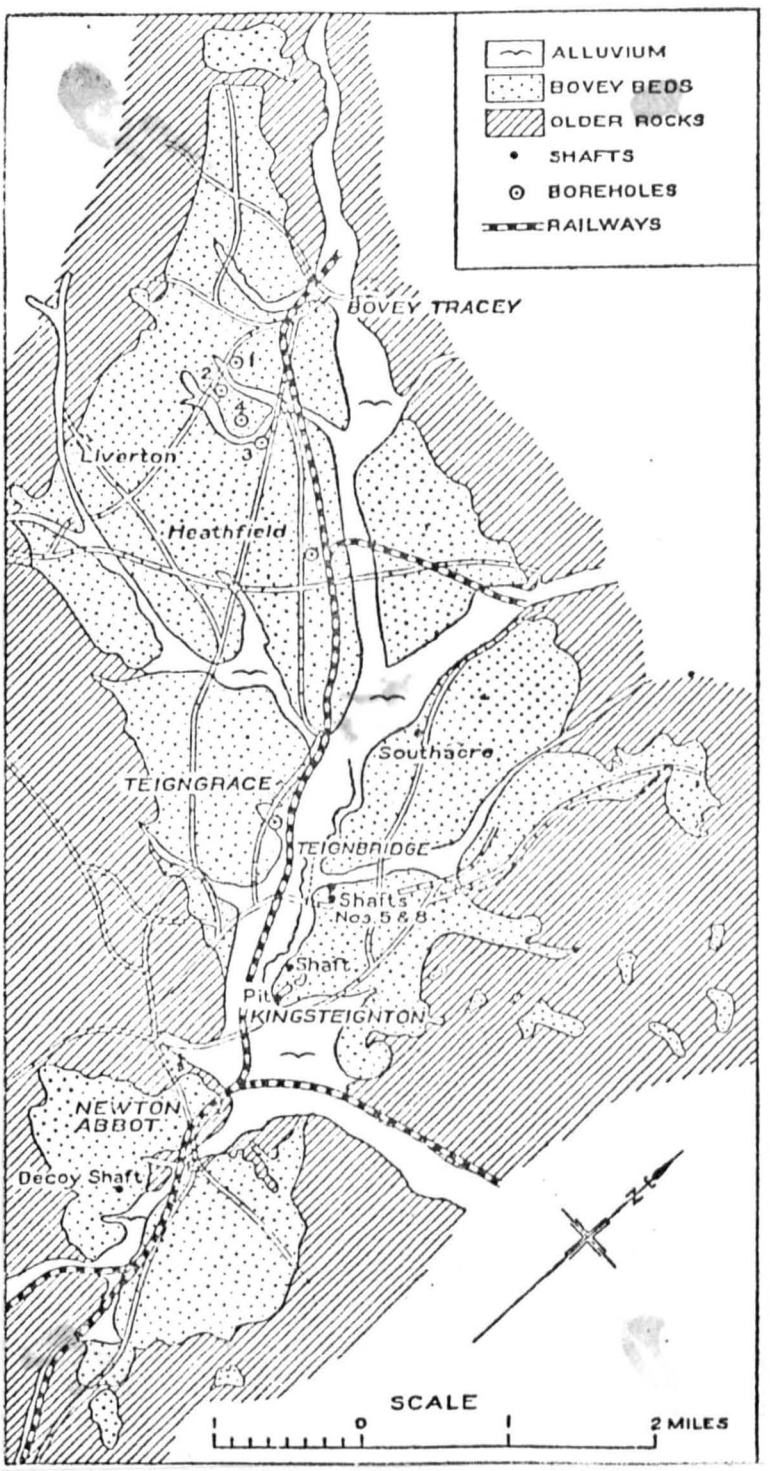
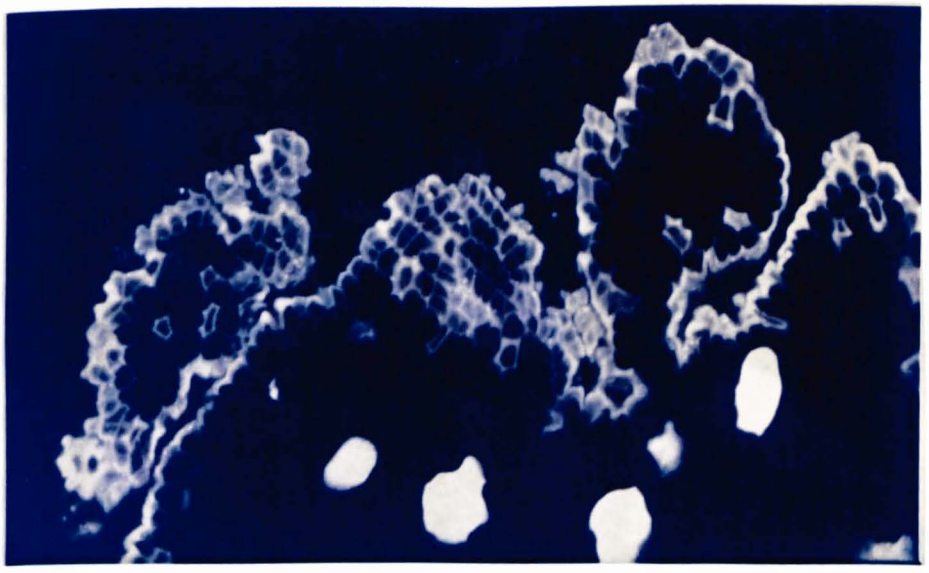
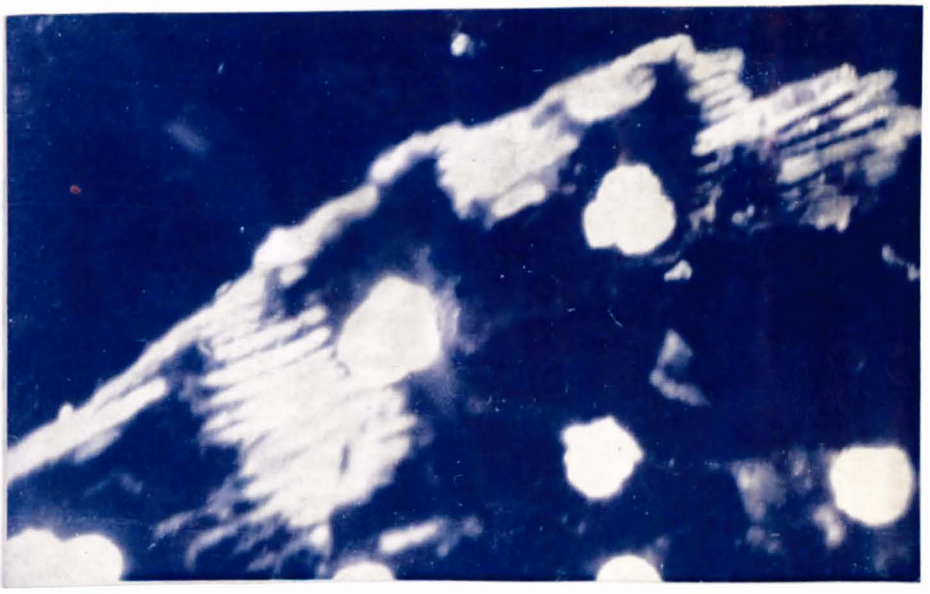


Figure 37. Fluorescent light microscopy of polished Bovey Tracey Lignite.



1 mm.



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fluorescence in ultra-violet light. The leaves are enclosed by a thick hypoderm (white in the photographs) which is seen incorporated with the cuticle. The inner structures of the leaves are less resistant to decay and there is no evidence of a woody vascular bundle. The resin bodies are extremely abundant and account for the high content of resinous material which may be separated from the montan wax of this lignite.

In the Order Coniferales, Table 10, most of the conifers are evergreen, but several extinct genera are deciduous e.g. Larix, Taxodium and Metasequoia[160]. In Taxodium and Metasequoia complete twigs fall each autumn. This characteristic is shown also by Sequoia couttsiae in the Bovey Beds, where the lignites show a profusion of twigs throughout the succession.

TABLE 10. THE CLASSIFICATION OF THE CONIFEROPHYTA

DIVISION	CONIFEROPHYTA	
ORDER	CORDAITALES (Fossil Order)	CONIFERALES
FAMILY		LEBRACEIACEAE, CUPRESSACEAE PODOCARPACEAE, PINACEAE, TAXODIACEA*, ARAUCARIACEAE, TAXACEAE and CEPHALOTAXACEAE.

* TAXODIACEAE (contains 10 genera including the genus Sequoia).

COLLECTION OF THE LIGNITE SAMPLE

The samples of lignite were collected from a large pit on an unclassified road three miles north-east of Newton Abbot. An unweathered sample was collected from a horizon in a total succession of about 75 feet, lying immediately above the "Big Coal".

CHAPTER VIII

LIPIDS IN THE BOVEY TRACEY LIGNITE

(a) INTRODUCTION

A knowledge of the lipid and terpenoid constituents of lignites is important in that it might allow us to understand the diagenetic processes that are responsible for causing the alteration of deposited organic material to petroleum-type organic compounds. Although little is known about the cell constituents, storage products and metabolites of marine organisms that contribute to the organic biomass of the ocean floor, quite an extensive literature is available concerning the natural product chemistry of terrestrial flora and fauna. Terrestrial accumulations of plant debris have contributed to lacustrine lake sediments and extensive deposits of peats, lignites and coals. If one assumes that plant biochemistry has not altered significantly throughout geological history, then it may be possible to relate organic compounds found in fossil plants with those of contemporary plants. This field of interest has been referred to as palaeochemotaxonomy. Prior to discussing the lipid and terpenoid constituents of peats and lignites, it is pertinent to review, briefly, the types and distributions of lipids in cuticular waxes.

Recently, Mazliak[161] has reviewed the chemistry of plant cuticles in which he has described the separatory techniques developed for the classification of cuticular wax constituents. An interesting remark that he has made is that many chromatographic techniques do not separate mixtures of natural wax esters with chain lengths greater than C_{32}. He has criticised the work of some authors, e.g. Warth[162], who stated that in natural wax esters, associated fatty acids and alcohols have similar chain lengths. In fact this supposition has now been shown to be incorrect[163]. Mazliak has noted several conclusions relating to the types and distributions of aliphatic lipids

in cuticular waxes, which are briefly stated as follows.

(i) different chemical classes are usually represented by a complete family of homologous compounds and frequently a major homologue can be found in each case.

(ii) no marked difference has been found between the fatty alcohols and acids, in cuticular waxes.

(iii) the chain lengths of the major wax constituents range from C_{14} to C_{35} and the CPI value of odd/even homologues of n-alkanes is much greater than unity, often by a factor of ten or more.

(iv) wax alcohols and acids generally have even carbon numbers. The fatty acids which are most abundant in a particular wax usually have shorter chain lengths than the alkanes or alcohols in the same wax; generally the chain length of the most abundant n-alkane is longer than that of the most abundant fatty alcohol.

Eglinton and Hamilton[164] have reported the chemotaxonomic applications of alkane distributions in plants as a method of species identification. More recent studies of the chemotaxonomic applications of leaf-wax alkanes to the subdivision of the Order Coniferales have been reported by Herbin and Robins[165] and del Castillo et al[166]. The former authors have reported that ω - hydroxy - alkanoic acids appear to be of value as chemotaxonomic markers, for the identification of species within the Pinus genus.

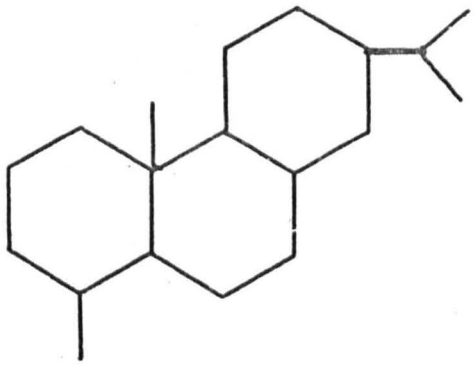
Work done by Eglinton et al[167] and Douglas and Eglinton[168] has drawn attention to fossil cuticular-wax constituents, of which the n-alkanes may prove to be of palaeochemotaxonomic importance. Wollrab et al[169] have shown that the CPI values of odd/even n-alkanes in lignites and brown coals are similar to those of contemporary plants. Brooks et al[170] have shown that normal, branched and cyclic saturated alkanes can be produced by the pyrolysis of Australian coals. Pyrolyses of long-chain

wax esters extracted from these coals yielded n-alkanes that showed no odd/even predominance.

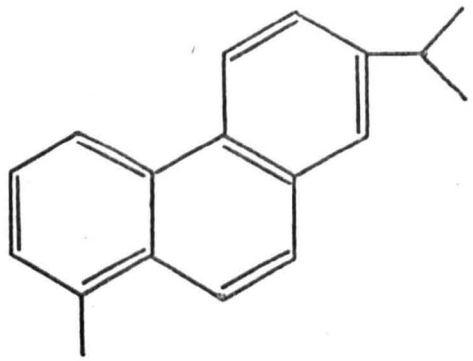
The occurrence of lipids and terpenoids in mosses, peats, lignites and brown coals is discussed below. A review of these constituents in peats has been reported by Howard and Hamer[171]. These authors have suggested that a comparison of the extracts from peat with those obtained from coal may give information about the origin of coal. The methods of extraction of waxes from peats are similar to those which yield montan wax from lignite. Several workers have separated crude peat extracts into asphalt, wax and resin fractions based on the method of Graefe[172]. These fractions have been defined in terms of their solubility in isopropanol. Thus asphalt is defined as the material insoluble in hot alcohol, wax as that part which is soluble in hot alcohol, but insoluble in cold alcohol, and resin as the part which is soluble in cold alcohol. This separatory scheme has been used as a preliminary step in the separation of crude montan wax extracts of lignites prior to further fractionation by column chromatography.

The occurrences of cyclic hydrocarbons bearing similarities in structures to that of abietic acid (Fig. 38), a diterpenoid acid of common occurrence in the order Coniferales, have been reported. Fichtelite (Fig. 38), a hydrocarbon which has been previously identified in fossil confiers, is thought to have been formed from abietic acid by hydrogenation and decarboxylation reactions[173]. Retene and iosene (Fig. 38) are often associated with fichtelite and Hoering[173] has suggested that the former two compounds have been produced either by reactions causing cyclisation to yield iosene or a sulphur rich de-hydrogenating environment that would produce retene. Brunn[174] has isolated dehydroabietane (Fig. 38) from fossil Scots Pine (5000 yr.) and he has suggested that it is the reduced product of abietic acid. Subsequently the same hydrocarbon was reported by Swan[175] in a forest

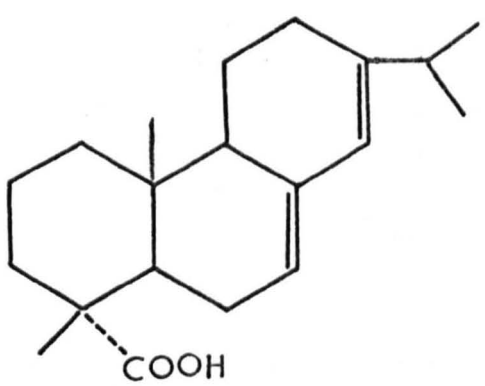
Figure 38



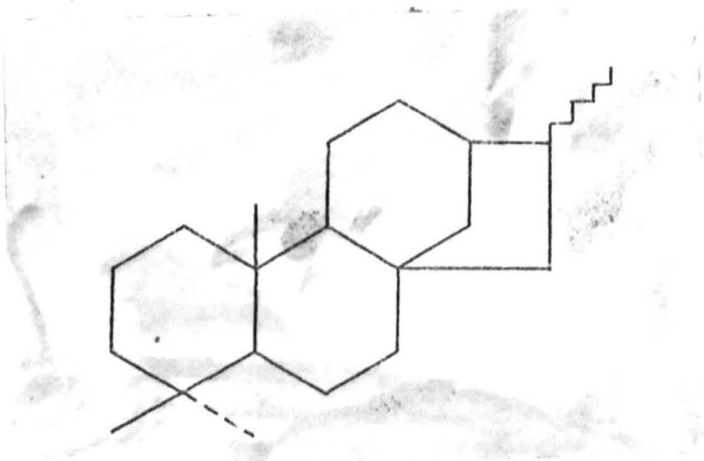
FICHTELITE



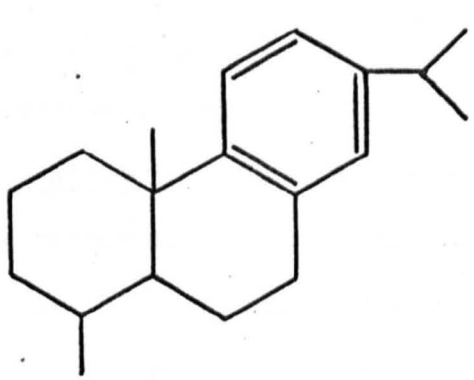
RETENE



ABIETIC ACID



IOSENE



DEHYDROABIETANE

soil from Bella Coole, British Columbia.

The occurrence of steroids and triterpenoids in a young peat has been described by McLean et al[176]. These workers saponified the light petroleum extract of Scottish peat, (Cumbernauld) and used alumina column chromatography to separate the unsaponifiable fraction. They identified friedelan-3 β-ol (Fig. 39) and other unknown triterpenoids. A mixture of sterols, whose physical constants were in agreement with those reported by Ives and O'Neill[177] for β-sitostanol, and α-sitosterol, isolated from Canadian peat-moss sphagnum were also identified. The presence of β-sitosterol (Fig. 39) in peats (Scottish peat moss) was previously reported by Black et al[178]. Ikan and Kashman[179] have also reported the presence of friedelin, β-sitosterol, β-sitostanol and friedlan-3 β-ol in an Israeli peat from Hula. These authors also briefly reviewed early literature on the occurrence of triterpenoids and steroids in lignites.

Since 1960, more extensive use of gas chromatographic techniques has afforded accurate information on the distribution of homologues within the various lipid classes. Morrison and Bick[180] have utilised g.c. in a study of the lipid constituents of peat wax isolated from phragmites (a marine plant) and have identified n-alkanes, n-fatty acids, n-methyl ketones, and n-fatty alcohols within the carbon range C_{16}-C_{35}. A recent short communication, by Marsili and Morelli[181], reports the occurrence of a triterpene hydrocarbon from mosses. These authors have reported a triterpene hydrocarbon, 22 (29)-hopene, (diploptene), Fig. 41 in Thamnium Alopecurum the most common Bryophyta of Italian flora. From g.c. retention data and i.r. spectroscopy they have identified ergosterol, stigmasterol and β-sitosterol. From a saponified extract they have established the presence of normal, saturated, and unsaturated fatty acids in the range C_{12} to C_{28}. The only other reports on the occurrences of triterpene hydrocarbons in plants are from ferns and lichens[182].

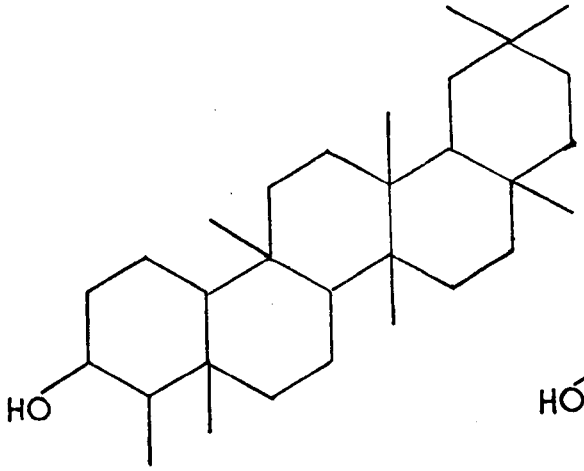
More extensive literature is available dealing with the lipid constituents of lignites. Wollrab[183] analysed Bohemian montan wax and found it to consist of a wax fraction (50%), a resin fraction (30%) and an asphalt fraction (19%). The wax fraction consisted of n-alkanes (2.2%), esters and alcohols (26.5%) and free acids (14.2%). The remaining substances in the wax fraction were not identified.

Jarolimek et al[184] have identified the n-alkanes occuring in a Bohemian brown coal. Although they reported the n-alkane homologues extending from C_{10} to C_{37}, with the even numbered alkanes predominating, Wollrab[34] later showed that the predominant n-alkanes were the odd numbered C_{29} (39.1%), C_{31}(27.5%) and C_{27}(13.9%) members. These authors were the first to report the presence of iso- and anteiso-branched alkanes in lignites. Edwards et al[185] noted that the hydrocarbons in an undefined montan wax were mainly the n-alkanes ranging from C_{23}-C_{33} with minor amounts of the C_{16}-C_{22} and C_{34}-C_{35} homologues. They also identified n-fatty acids in the C_{16}-C_{35} range and n-fatty alcohols in the C_{10}-C_{21} range.

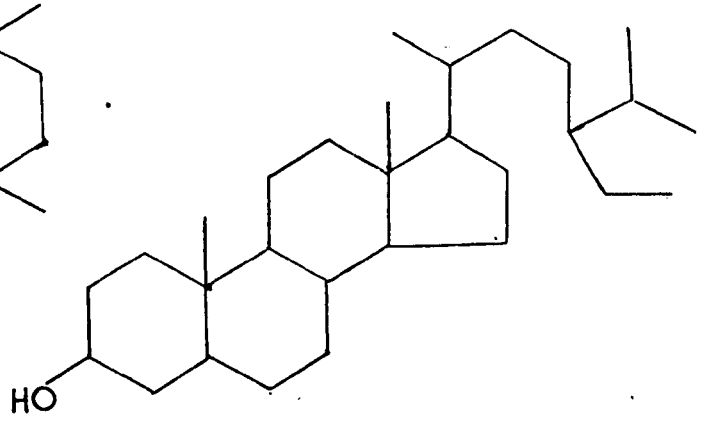
Jarolim, et al[186] have identified normal fatty alcohols (C_{22}, C_{24}, C_{26}, C_{28}, C_{30} and C_{32}) in Bohemian montan wax. In contrast to Wollrab's work, they have reported that the free acid content is greater than the ester and alcohol content. This may be due to the difficulty in isolating, quantitatively, natural esters by column chromatography, due to their low solubility in cold non-polar solvents. They established that the dominant fatty acid occuring in both the free and esterified moieties was montanic acid (n C_{28}). Wollrab et al[169] have also reported the C_{22}-C_{34} fatty acids in Bohemian montan wax, supporting previous results of Hewitt et al[187].

Recently, Wollrab[183] has reported the branched-chain, iso- and anteiso-fatty acids in Bohemian montan wax although the writer believes that the resolution of the gas chromatographic columns which were

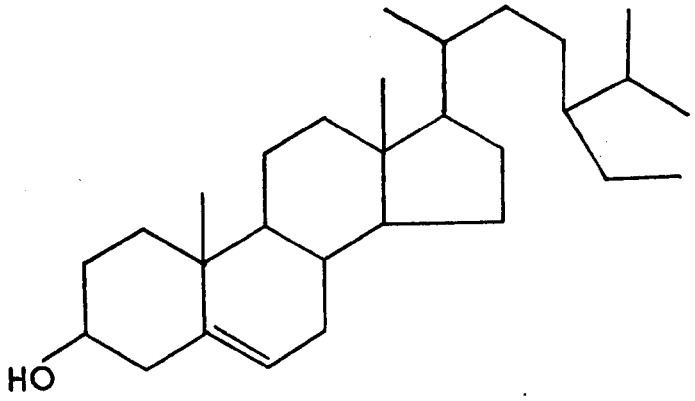
Figure 39



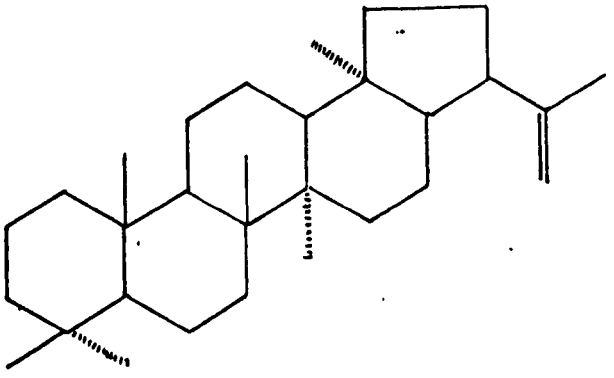
FRIEDELAN 3β OL



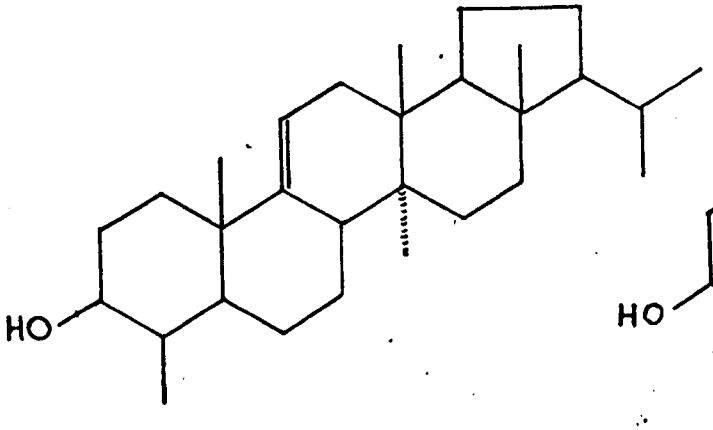
β-SITOSTANOL



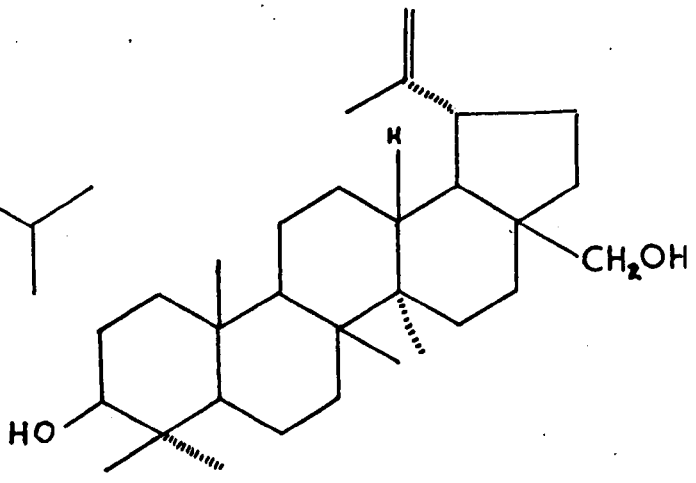
β-SITOSTEROL



22 (29)-HOPENE

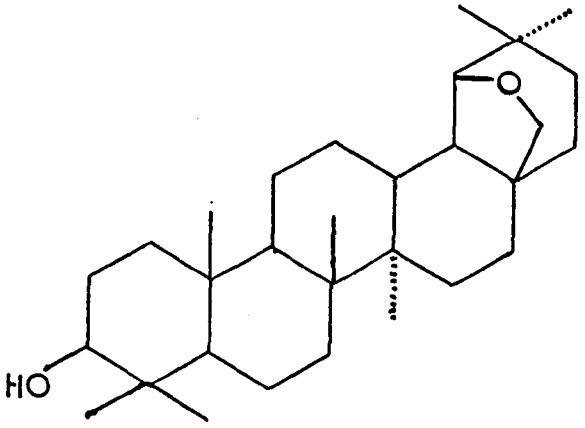


ISOARBORINOL

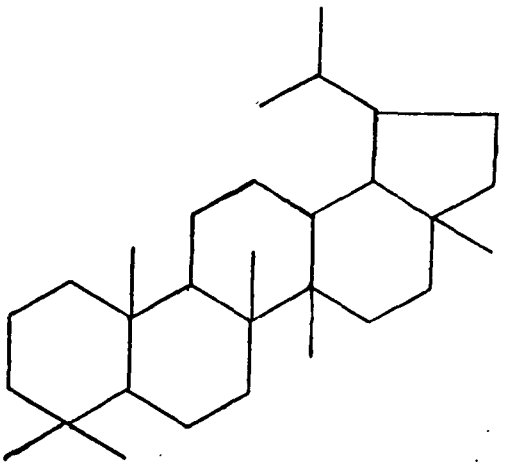


BETULIN

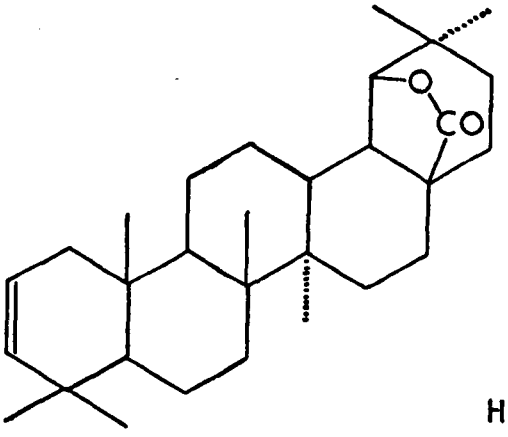
Figure 40



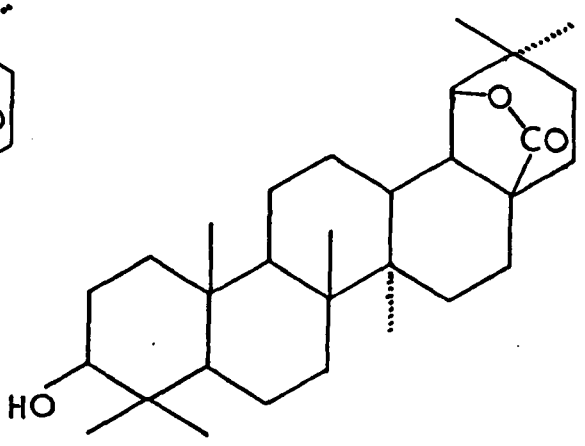
ALLOBETULIN



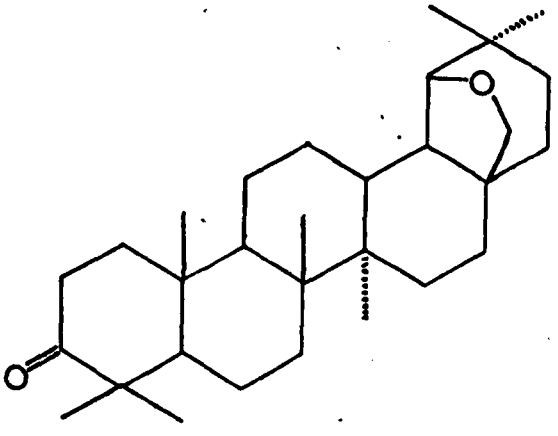
LUPANE



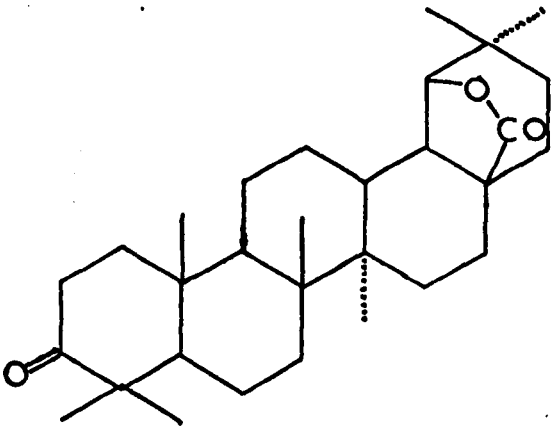
OXYALLOBETUL-2-ENE



OXYALLOBETULIN

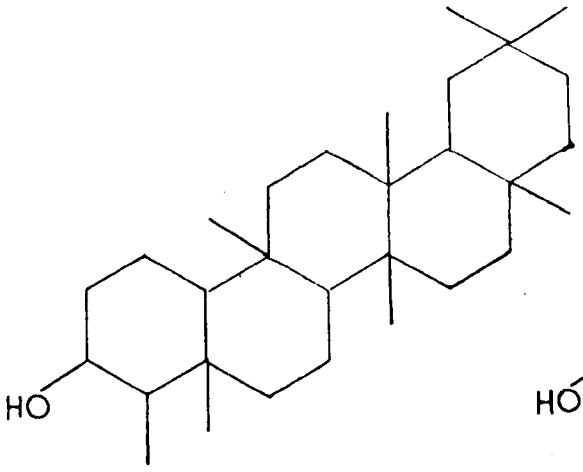


ALLOBETULONE

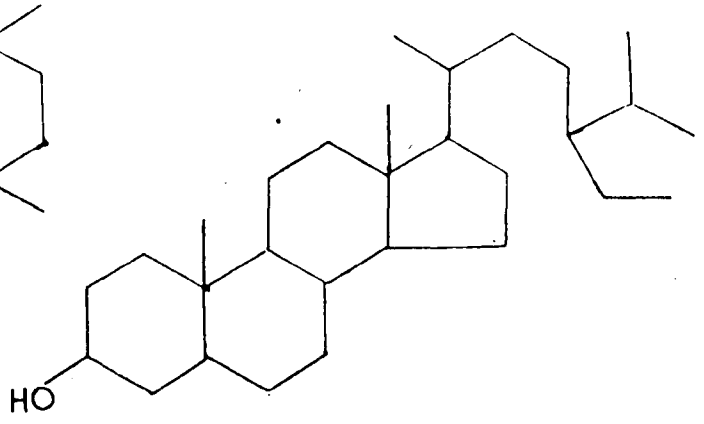


OXYALLOBETULONE

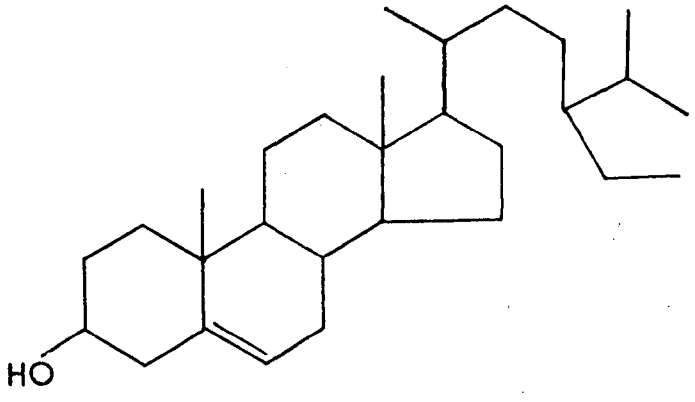
Figure 41



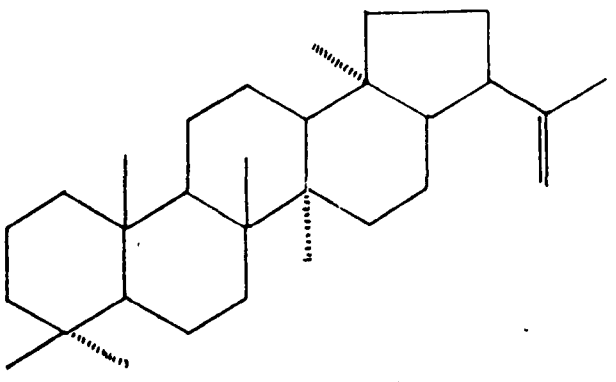
FRIEDELAN 3β OL



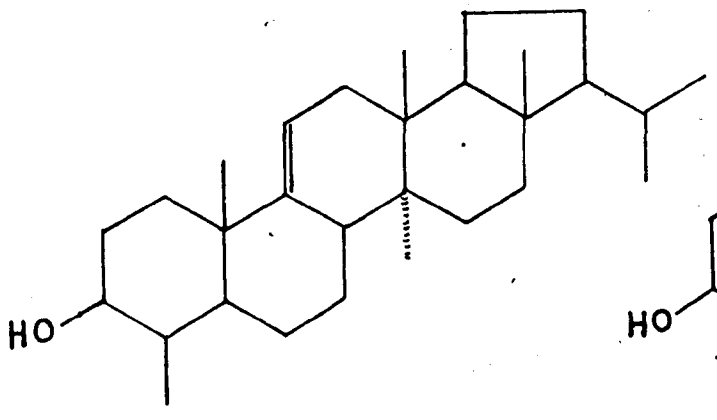
β-SITOSTANOL



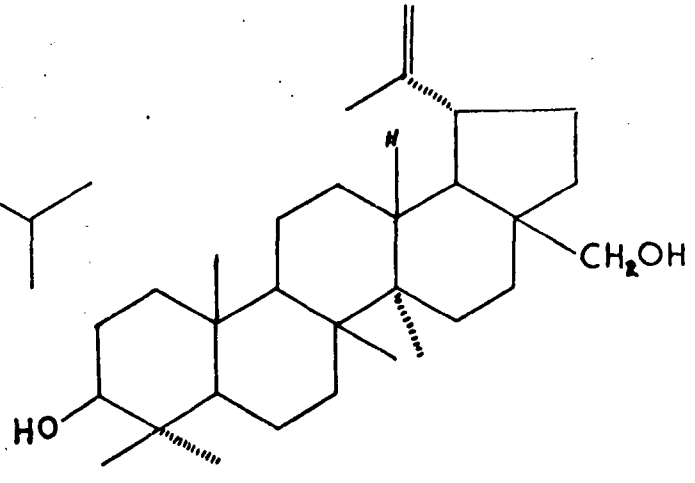
β-SITOSTEROL



22 (29)-HOPENE



ISOARBORINOL



BETULIN

employed were not high enough to determine the position of branching.

Albrecht et al[188, 189] have isolated a pentacyclic unsaturated alcohol from the Messel oil shale (ca. 50 x 10^6 yr. old) which they identified as isoarborinol, Fig. 41. These authors consider that this oil shale has never been buried to a depth greater than three hundred metres, and consequently it has not been exposed to a high-temperature environment which might have caused dehydrogenation and dehydration. They also noted that isoarborinol has only been isolated from tropical plants, and that the fossil flora found in the Messel Shale are closely related to the contemporary flora of South East Asia.

Ruhemann and Rand[190] were able to separate and identify the triterpenoids, betulin and allobetulin (Figs. 40 and 41) from an extract of a German lignite. Later work has confirmed the presence of triterpenoids structurally related to the lupane series (Fig. 40) both in lignites and shales. Thus, Carruthers and Cook[191] have employed column chromatography to isolate a triterpene from a high boiling fraction of petroleum and Barton et al[192], by comparison of this triterpene with an authentic specimen, were able to identify it as oxyallobetul-2-one (Fig. 40). Ikan and McLean[193] identified, in the unsaponifiable wax extract of Bovey Tracey lignite, the following members of the lupeol series; betulin, allobetulin, oxyallobetulin, allobetulone and oxyallobetulone (Figs. 40 and 41).

An extensive study of triterpenoids and related compounds, occurring in a North Bohemian lignite from the "Josef Jan" mine, has been initiated by Jarolim et al[184,186,194,195]. These authors chromatographed an extract of this lignite on alumina into several fractions which were then re-chromatographed into several hundred sub-fractions. Some of these sub-fractions were crystallised and yielded over thirty individual components, many of which were structurally identified (shown in Table 12). The completeness of this analysis has made it possible to understand some of the chemical diagenetic processes that have taken place during

TABLE 11. TRITERPENOIDS IN BOHEMIAN LIGNITE.

Number	Compound	M.P. (°C)	Formula	$[\alpha]_D^{20}$	Functional groups
	?	227	$C_{30}H_{52}$	−28.8	hydrocarbon
XXXI	Octahydro-2,2,4a,9-tetramethylpicene	233–235	$C_{26}H_{30}$	−34.2	aromatic hydrocarbon
XXXII	α-Apoallobetulin	203–206	$C_{30}H_{48}O$	+65.5	unsaturated ether
XXXIII	Allobetul-2-ene	249–250.5	$C_{30}H_{48}O$	+47.6	unsaturated ether
XXXIV	Tetrahydro-1,2,9-trimethylpicene	230–231.5	$C_{25}H_{24}$	+50.0	aromatic hydrocarbon
	?	275–275.5	$C_{25}H_{24}$	+146.1	aromatic hydrocarbon
	?	204–205	$C_{25}H_{24}$		aromatic hydrocarbon
XXXV	Tetrahydro-2,2,9-trimethylpicene	251–252	$C_{25}H_{24}$	0.0	aromatic hydrocarbon
XXIX	Friedelin	253–255	$C_{30}H_{50}O$	−21.6	ketone
XXXVI	1,2,9-Trimethylpicene	277	$C_{25}H_{20}$		aromatic hydrocarbon
XXXVII	23,25-Bisnormethyl-2-desoxyallobetul-1,3,5-triene	261	$C_{28}H_{40}O$	+8.4	aromatic ether
XXXVIII	α-Apooxyallobetul-3-ene	289–291	$C_{30}H_{46}O_2$	+70.3	unsaturated lactone
XXVIII	Oxyallobetul-2-ene	363	$C_{30}H_{46}O_2$	+75.2	unsaturated lactone
XXXIX	23,24,25,26,27-Pentanormethyl-2-desoxyallobetul-1,3,5,7,9,11,13-heptaene	250	$C_{26}H_{28}O$	+150.8	aromatic ether
XXX	Friedelan-3β-ol	285–286	$C_{30}H_{52}O$	+20.7	alcohol
XL	Allobetulone	228–229	$C_{30}H_{48}O_2$	+84.4	ketoether
XLI	Friedelan-3α-ol	310	$C_{30}H_{52}O$	+24.2	alcohol
XLII	3-Dehydrooxy-allobetulin	338	$C_{30}H_{46}O_3$	+91.3	ketolactone
XXVII	Allobetulin	266	$C_{30}H_{50}O_2$	+50.7	hydroxyether
XLIII	Oxyallobetulin	347	$C_{30}H_{48}O_3$	+46.0	hydroxylactone
XXVI	Betulin	254–255	$C_{30}H_{50}O_2$	+26.4	unsaturated diol
	?	330			trisubst. double bond, polyalcohol
	?	267–269	$C_{30}H_{50}O_2$		hydroxyketone
	?	196–198	$C_{30}H_{52}O_4$		
XLIV	Ursolic acid	285–287	$C_{30}H_{48}O_3$	+63.3	carboxylic acid
	?	253–256	$C_{30}H_{50}O_3$	+83.8	polyalcohol

the coalification of this lignite. The alteration of plant tissue to peat and then lignites has probably proceeded in an anaerobic environment producing an acid - reducing environment. The triterpenoids which are structurally related to the betulins could possibly have been formed through acid-induced isomerisation of the lupene series. Friedelane derivatives (rearranged oleananes) and trimethyl picenes related to the ursane series are also present.

Another important factor is that under the reducing and dehydrogenating conditions prevalent during diagenesis of the plant tissue, a gradual aromatisation of the triterpenoid rings has taken place. This aromatisation has proceeded through rings A, B, C and D until some of the triterpenoid substances have become completely aromatised, forming methyl picenes.

(b) LIPIDS IN THE BOVEY TRACEY LIGNITE

Lignites have been defined on the basis of several properties which serve to distinguish them from coals. Elemental carbon analyses show that lignites contain between 60% and 71% vitrite carbon[196] (derived from wood tissues) and microscopical examination reveals the presence of recognizable plant structures as shown in Fig. 37.

Lignites are mainly found in strata of Recent origin. The largest accumulations of lignites were deposited during the Oligocene and Miocene epochs ($26 - 38 \times 10^6$ yr) of the Tertiary Period. There are only a few examples of lignites that occur in earlier Periods of geological history, e.g. the Russian "papercoal" which is Carboniferous in age[197]. Lignites are important sources of fuel and are commercially important since they yield, on benzene extraction, montan wax, which is extensively used in several industries.

The montan wax extract of the Bovey Tracey lignite has been studied previously by Ikan and McLean who isolated several triterpenoids and the n-fatty alcohol, tetracosanol. The work that is discussed in this

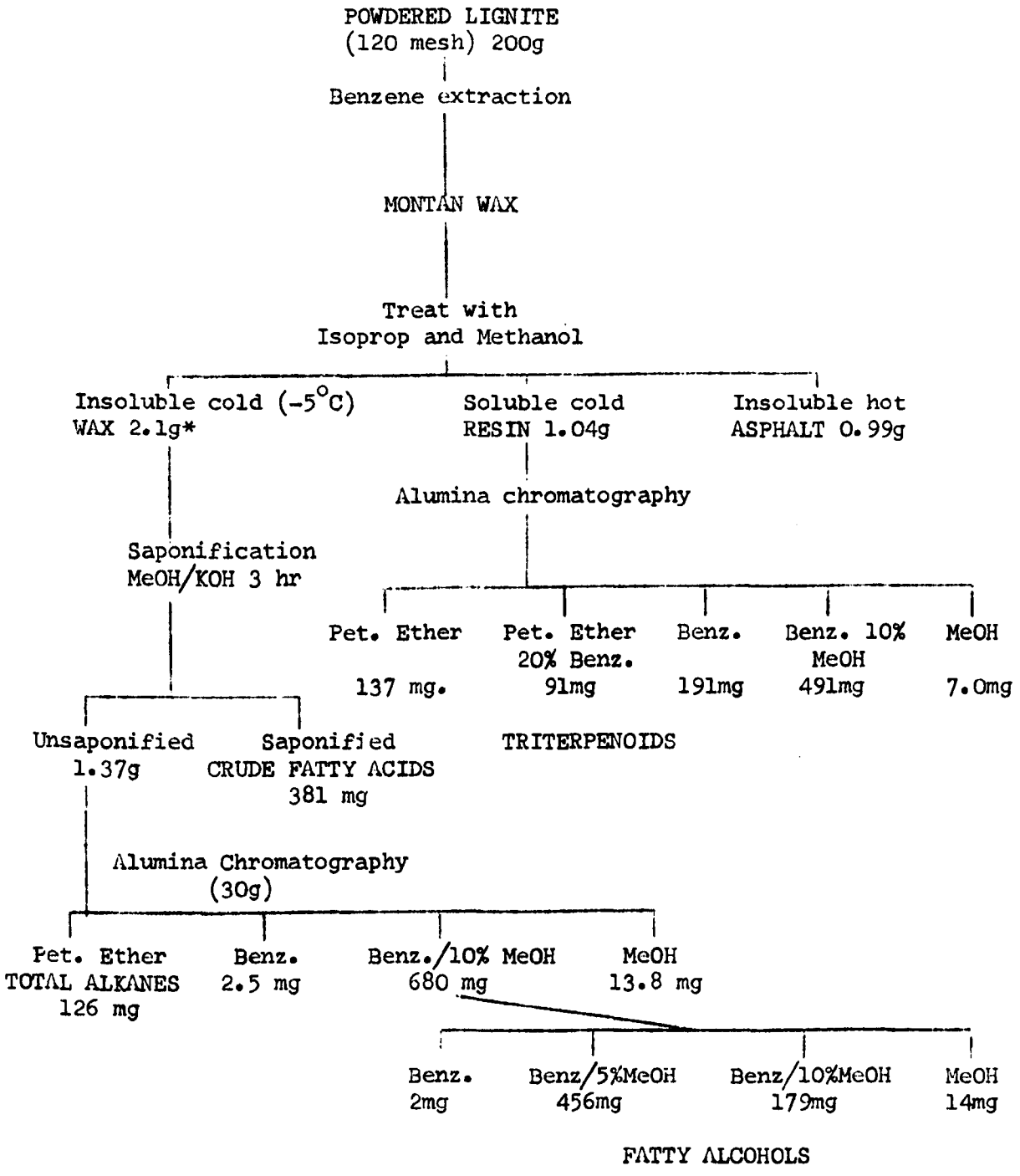
chapter describes the lipid content of this lignite more thoroughly, confirms the presence of the triterpenoids reported by the above authors and the tentative identification of the triterpenoid, lupeol, not previously reported in the sedimentary environment.

A benzene extract of the powdered lignite yielded a brown montan wax after evaporation of solvent (2.15% by weight). This montan wax was fractionated, according to the method of Graefe, into a wax, a resin and an asphalt fraction. Saponification of the wax, afforded a neutral fraction (63.2%) and an acidic fraction (18.1%). The former fraction was chromatographed on an alumina column (grade 4) using the following elution scheme; petroleum ether, benzene, benzene-methanol (90:10) and methanol. The eluants were monitored by infra-red spectroscopy and were shown to consist predominantly of saturated hydrocarbons (total alkanes) and fatty alcohols. The acidic fraction was methylated and the crude esters were chromatographed on alumina, using benzene as eluant. The product, after evaporation of solvent, was analysed by i.r., t.l.c. and g.c., and was shown to consist of long chain n-fatty acid methyl esters. A schematised "flow-diagram" for the whole procedure is shown in Table 12.

Hydrocarbons in the Bovey Tracey Lignite

Infra-red analysis and argentaceous t.l.c. showed that only saturated hydrocarbons were present in the total alkane fraction. Treatment of the total alkanes with 5A molecular sieve afforded, after HF dissolution of the sieve, normal and branched-cyclic alkane fractions. Separation of the n-alkanes by g.c. and identification by coinjection with standards, indicated the presence of a homologous series; this ranged from n-C_{17} to n-C_{33} with a maximum concentration at n-C_{27}, as shown in Fig. 42. There is a marked predominance of the odd membered homologues, and the CPI index in the range C_{22} to C_{33} is 2.6. These findings are similar to those of Wollrab and Streibl[183] for the Bohemian lignite,

TABLE 12. GENERAL PROCEDURE FOR THE ISOLATION OF LIPIDS FROM BOVEY TRACEY LIGNITE



* INSOLUBLE COLD REFINED WAX 2.10g

Sat. Hyds (incl Triterpanes) 126mgs	Fatty Acids 381 mgs	Fatty Alcohols 635 mgs

Fig. 42

Saturated hydrocarbons in the Bovey Tracey lignite

A. Normal alkanes

Chromatographic conditions: see Fig. 7.

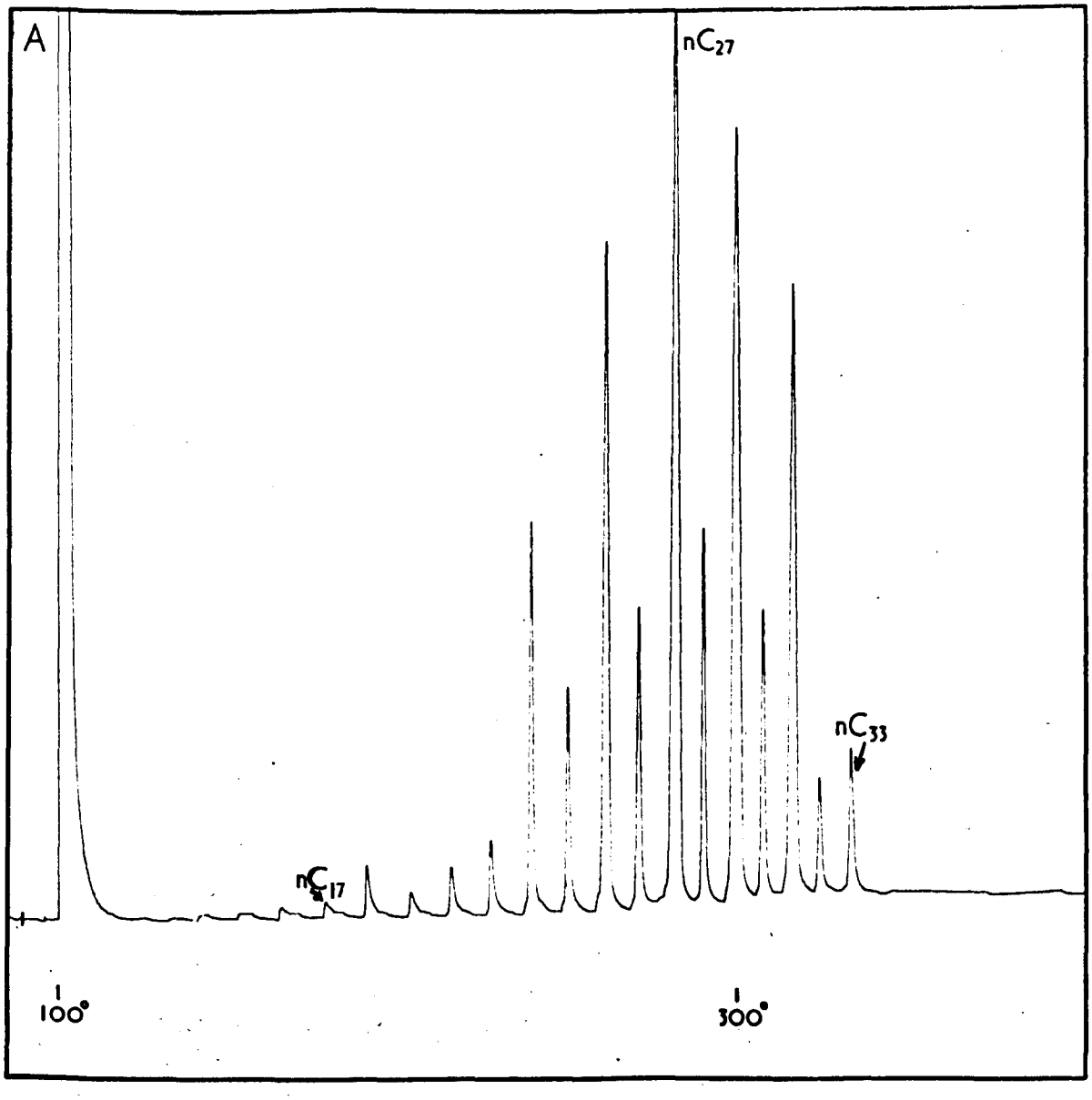

A
nC27
nC33
nC17
100°
300°

Fig. 43

Saturated hydrocarbons in the Bovey Tracey lignite

B. Total alkanes

Chromatographic conditions: see Fig. 7.

C. Branched-cyclic alkanes

Chromatographic conditions: as above.

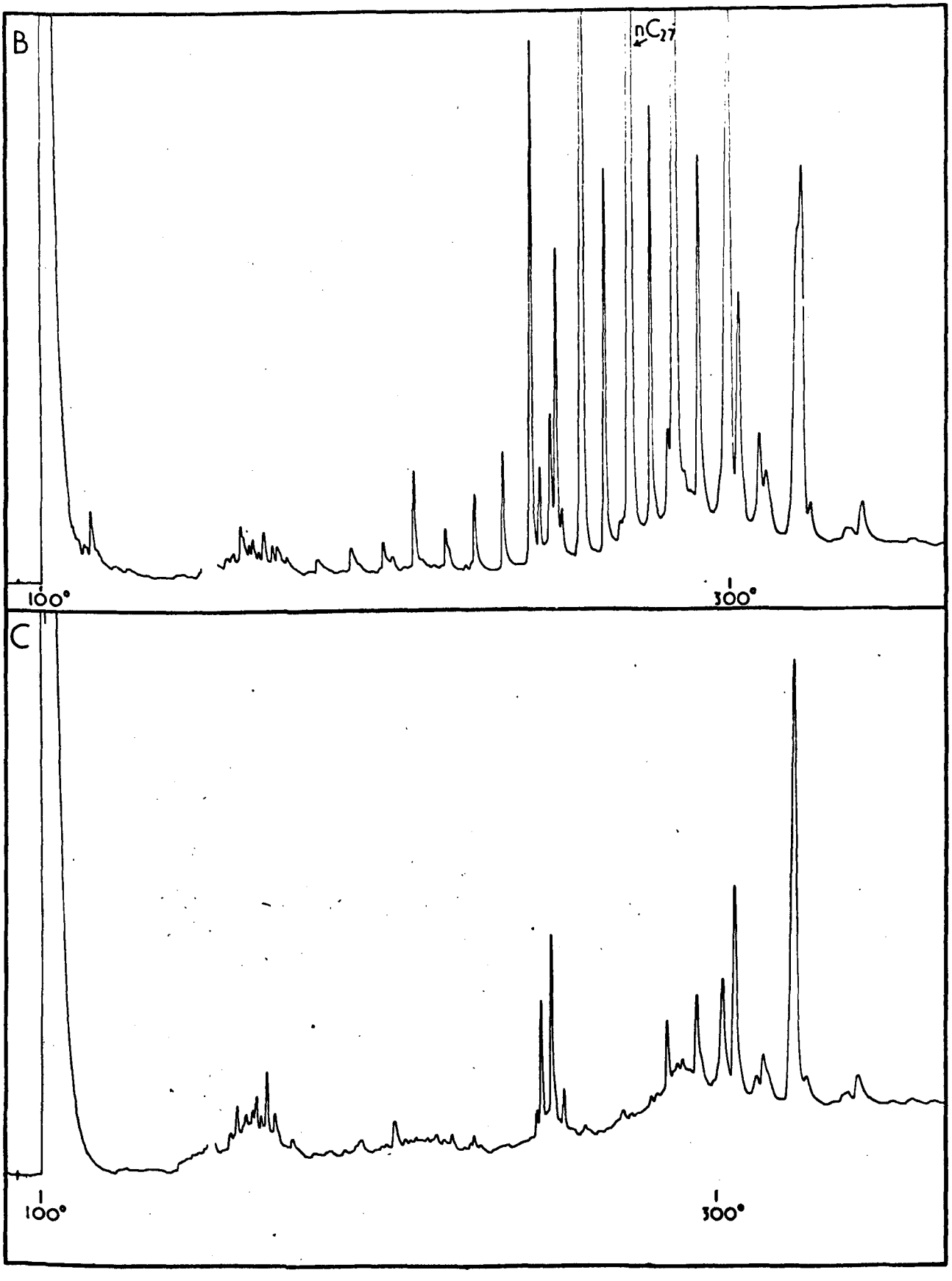
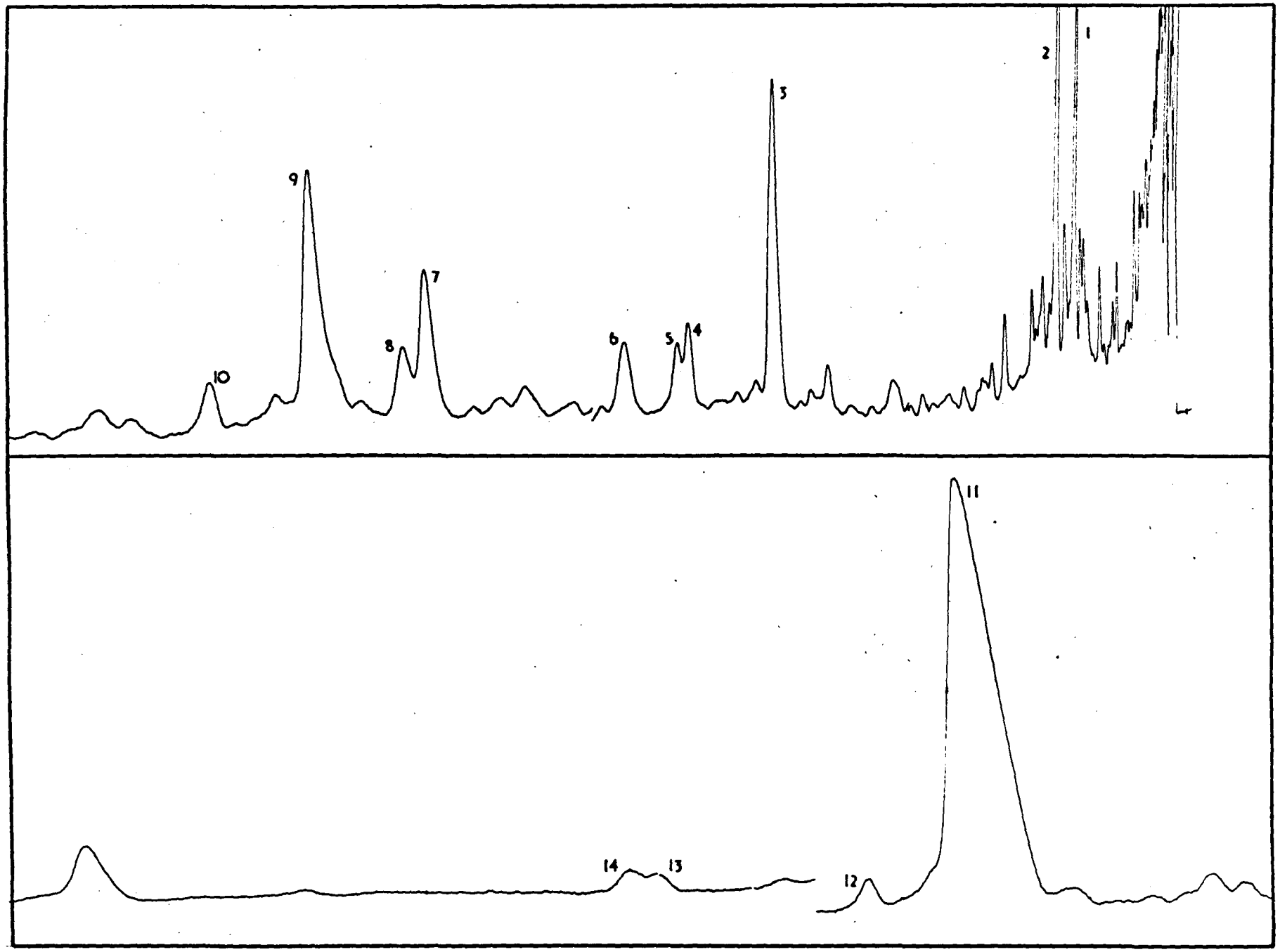

B
nC27
100°
300°
C
100°
300°

Fig. 44

Branched-cyclic hydrocarbons from the Bovey Tracey lignite showing polycyclic hydrocarbons, numbered 1 to 14 on the as chromatogram

Chromatographic conditions: stainless steel capillary column 200 feet x 0.010" I.D., coated with a 10% solution of PPE-7. The column was operated isothermally at 250°C, using nitrogen as carrier gas, 2 ml/min, with a split ratio of 8/1. Injector temperature, 300°C; detector temperature, 260°C.


1
2
3
4
5
6
7
8
9
10
11
12
13
14

and are in marked contrast to n-alkane distributions in ancient sediments, as exemplified above in the Marl Slate, Kupferschiefer and Caithness shales. The percentage abundances of the individual homologues in the n-alkane fraction are recorded in Table 13.

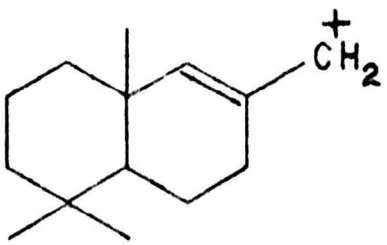
The gas chromatograms of the total and branched-cyclic alkanes are shown in Fig. 43. In the total alkane chromatogram it is evident that there are two areas which contain components with retention indices intermediate in value with those of adjacent n-alkane peaks. These branched-cyclic alkanes occur in the range $\underline{n}C_{23}$ to $\underline{n}C_{25}$ and $\underline{n}C_{28}$ to $\underline{n}C_{33}$. A third region of complexity occurs at the low molecular weight end of the chromatogram. No significant amounts of the isoprenoid hydrocarbons, pristane and phytane were found; this is in marked contrast to their almost ubiquitous occurrence in older shales. Kovats retention indices of the branched-cyclic alkanes were determined from runs with a g.c. capillary column (isothermal 250°) coated with a 10% solution of PPE-7 (7 ring polyphenyl ether). The gas chromatogram is shown in Fig. 44. Retention indices of the prominent components, numbered 1 - 14 in the chromatogram, are recorded in Table 14. By comparison of the peak intensities in Figs. 43 and 44, it was possible to obtain retention data, for the four most abundant components, on two liquid phases (PPE-7 and OV-1). It was not possible to correlate peak number 3 in Fig. 44 with a component in Fig. 43 since three possible peaks in the range C_{28} to C_{31} were of roughly equivalent intensities.

Combined g.c. - m.s. data of the high molecular weight hydrocarbons were very kindly recorded by Dr. A. McCormick*. Separations were carried out on a SCOT (support coated open tubular) column coated with PPE-7 and the spectra were recorded on an A.E.I. MS 12 mass spectrometer. Peaks numbered 1 and 2 (Figs. 44 and Table 14), gave

---

* A.W.R.E. Aldermaston.

the mass spectra shown in Fig. 45, A and B respectively. Spectrum A shows a prominent molecular ion at m/e 274, and the fragment ion at m/e 189 suggests that the hydrocarbon is a tricyclic diterpene with a molecular formula $C_{20}H_{34}$. A fragment ion at m/e 191 is characteristic of the fragmentation pattern of a pentacyclic triterpane and may be formally written as:



The fragment at m/e 189 suggests that an extra double bond is present in either of the ring systems. A prominent fragment ion at m/e 259 (M - 15) is due to the loss of a methyl group from the molecular ion, but it is not possible to specify the positions of methyl substituents in the molecule. The absence of a prominent ion at M - 43 shows that there is no isopropyl substituent in the molecule. The evidence therefore indicates that component 1 in table 14 is a tricyclic diterpene ($C_{20}H_{34}$) with 6 methyl substituents. A possible structure is shown here with a proposed fragmentation pattern.

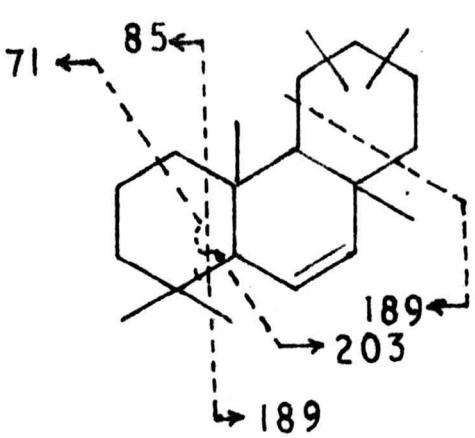
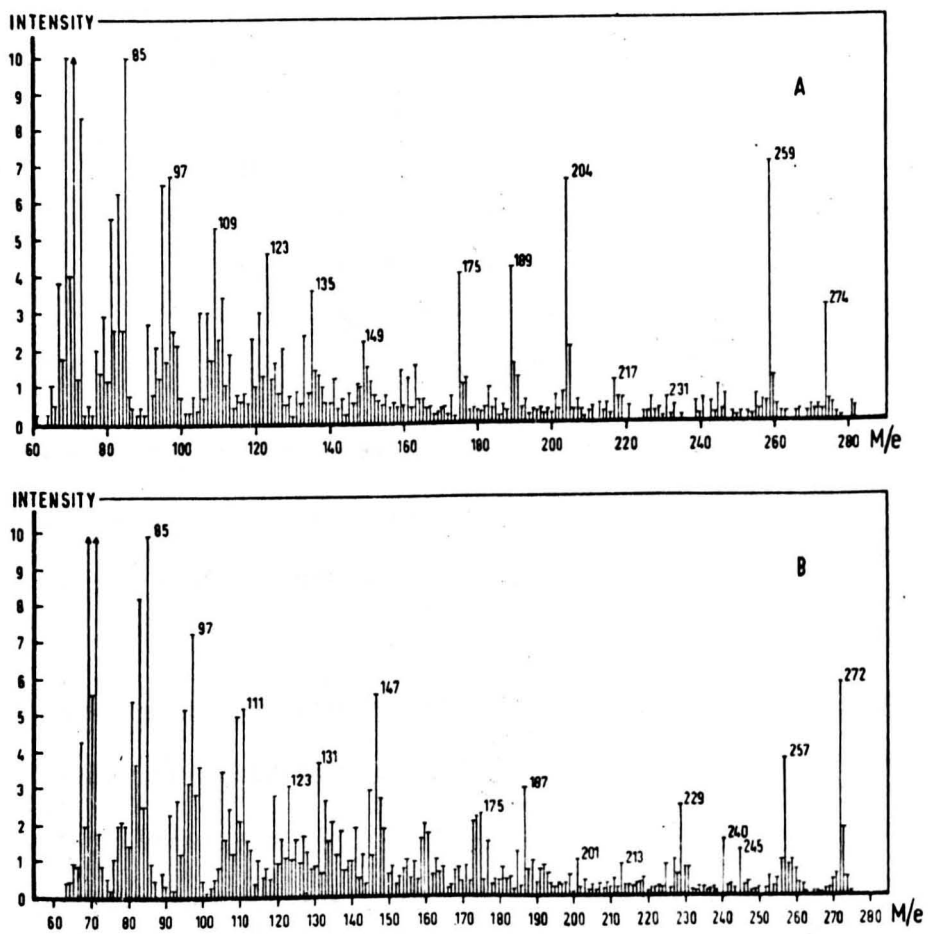
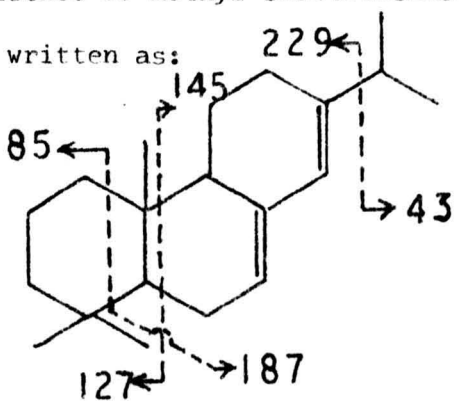


Figure 45


INTENSITY
A
85
97
109
123
135
149
175
189
204
217
231
259
274
M/e
INTENSITY
B
85
97
111
123
131
147
175
187
201
213
229
240
245
257
272
M/e

Spectrum B is similar to A except that the molecular ion at m/e 272 indicates a molecular formula of $C_{20}H_{32}$. The prominent ion at m/e 187 suggests that two double bonds are present in the molecule and a prominent M-43 ion at m/e 229 indicates the presence of an isopropyl substituent. A prominent M-15 ion indicates the presence of methyl substituents. A possible structure for this component may be written as:



Such a structure would be identical to that derived by reduction of the carboxylic acid group of abietic acid. Such a comparison, however, remains speculative since the mass spectra alone cannot unequivocally determine the structure.

Mass spectra were also recorded for higher molecular weight components in the Bovey Tracey branched-cyclic alkanes. Figs. 46 and 47 show six spectra, A to F. Unfortunately it has proved impossible to assign these spectra to specific components present in the chromatogram, Fig. 44, due to the fact that the relative retention indices of peaks eluted from the SCOT column were different from those recorded on the capillary column. Also, since the six components gave roughly equivalent intensities, it was not possible to directly correlate the component intensities with those present in Fig. 44. Nevertheless, it was possible to determine that nine components were present, spectra C, D and E containing two molecular ions (M) each, as shown. The molecular formulae of these hydrocarbons are shown in Table 15. Thus, spectrum F could represent a tetracyclic saturated

Figure 46

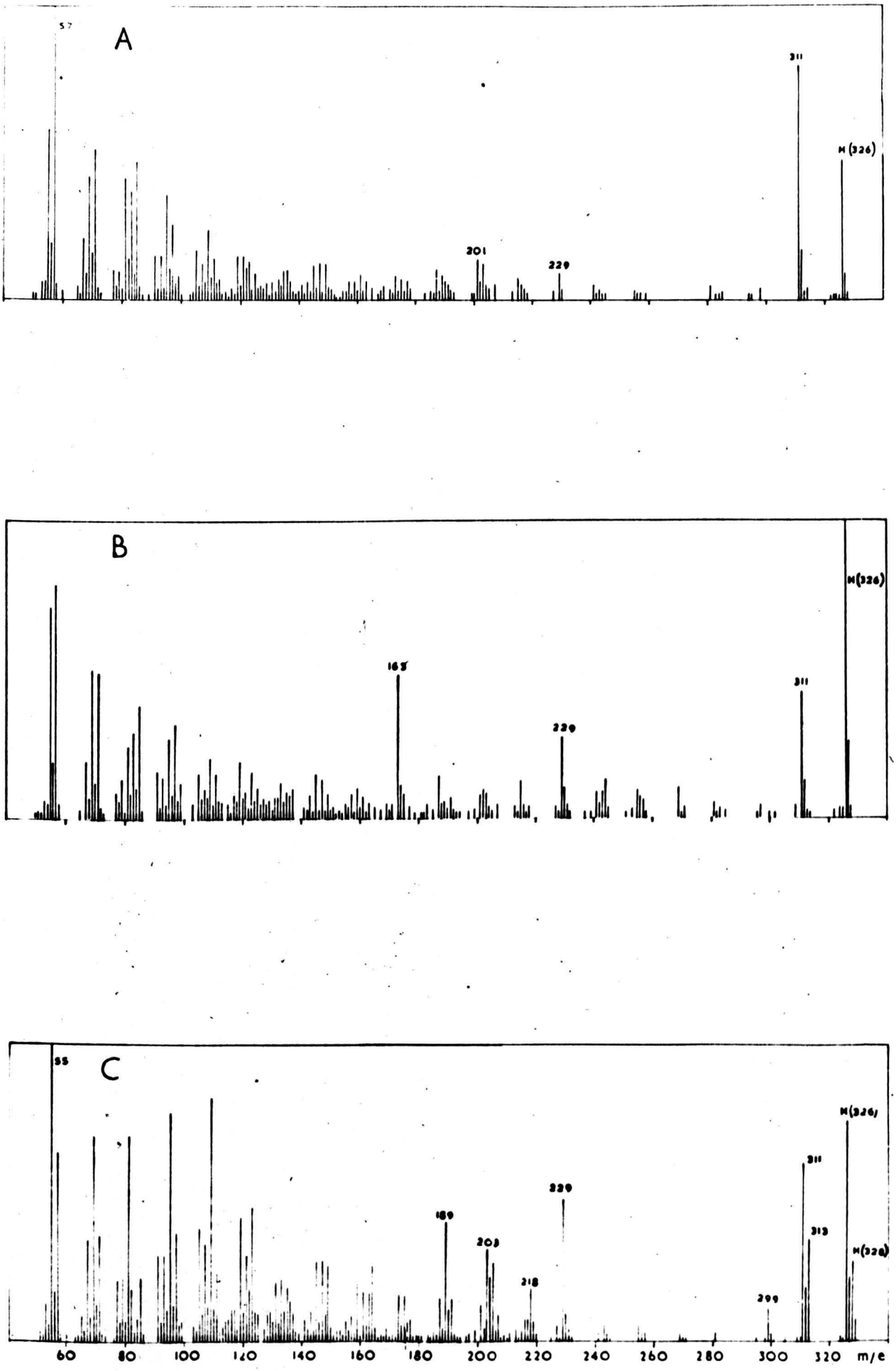
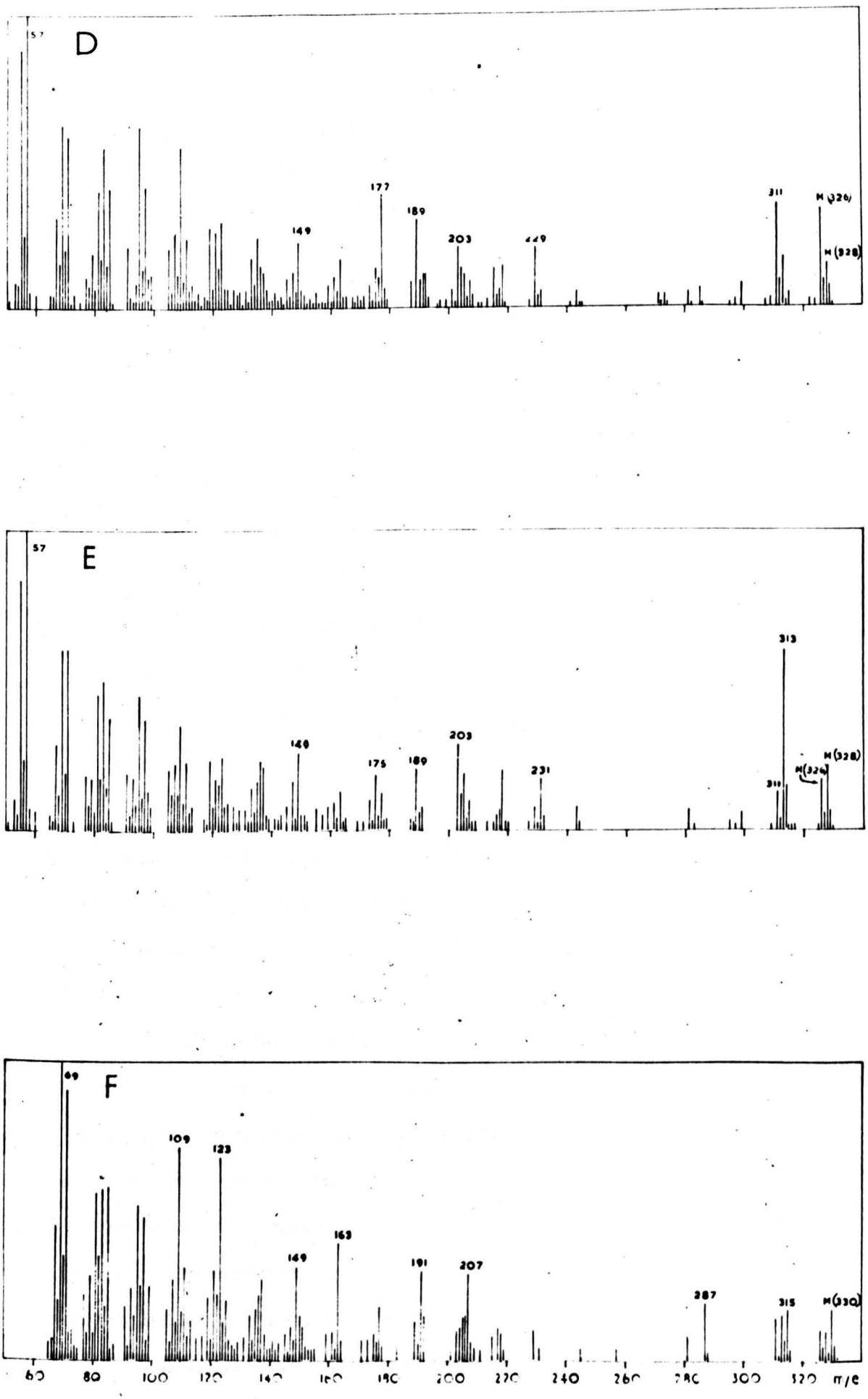

A
57
311
M(326)
201
229
B
M(326)
165
311
229
C
55
M(326)
311
229
189
313
203
M(328)
218
299
60
80
100
120
140
160
180
200
220
240
260
280
300
320
m/e

Figure 47


D
57
149
177
189
203
229
311
M(326)
M(328)
E
57
149
175
189
203
231
310
313
M(324)
M(328)
F
69
109
123
149
163
191
207
287
315
M(330)
60
80
100
120
140
160
180
200
220
240
260
280
300
320
m/e

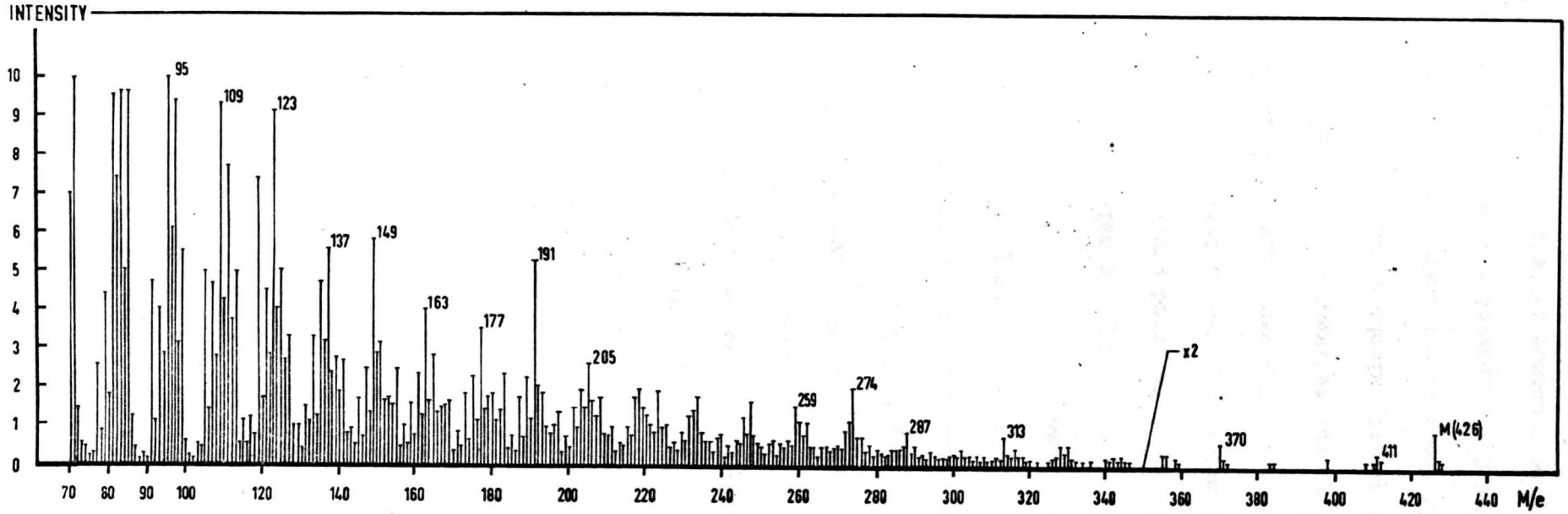
hydrocarbon containing an isopropyl substituent (prominent M-43 ion); three methyl substituents in rings A and B, would account for the prominent ion at m/e 191. The molecular ions at m/e 328 and 326 represent tetracyclic hydrocarbons containing one and two double bonds in their structures respectively.

Although it has been noted previously that i.r. spectrophotometry and argentaceous t.l.c. did not indicate the presence of unsaturated hydrocarbons, it should be stated that,

(1) if only minor amounts of unsaturated hydrocarbons are present, it is difficult to decide whether their olefinic bands would show in the i.r.

(2) due to the conformation of the triterpane ring system, i.e. all trans-chair conformation, it may be difficult for a "hindered" double bond to approach the silver ions on the surface sufficiently to interact. If this happens, the Rf value of a compound containing a sterically hindered double bond may be similar to that of the saturated compound.

The component numbered 11 in Table 14 was found to amount to 80% of the total branched-cyclic alkanes of this lignite. The mass spectrum of this component, illustrated in Fig. 48 was once again recorded by Dr. A. McCormick. Accurate mass measurement of the molecular ion at m/e 426 showed that it had a molecular formula of $C_{31}H_{54}$ and contained an extra methyl substituent. The presence of a fragment ion at m/e 191 indicates that the compound may be pentacyclic triterpane containing nine methyl substituents. On the basis of mass spectrometry alone, it is not possible to unequivocally assign the positions of the methyl substituents. However, it is possible to ascertain, on the basis of the fragment ion at m/e 191, that rings (A and B) or (D and E) contain three methyl substituents. Although no triterpanes exist in nature, the presence of such compounds in sediments, containing 31 and 32


INTENSITY
10
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3
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1
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95
109
123
137
149
163
177
191
205
259
274
287
313
x2
370
411
M(426)
70
80
90
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
M/e

carbon atoms has been reported previously[50]. It is interesting to note that a triterpenoid acid, commic acid-A ($C_{31}H_{50}O_4$), has been also reported in the plant family Burseraceae[196].

Fatty alcohols in the Bovey Tracey Lignite

The unsaponified wax fraction (see Table 12), after chromatography on alumina, was analysed by i.r., t.l.c. and g.c., and was found to consist predominantly of long chain saturated fatty alcohols. The fatty alcohols which were recovered amounted to a concentration of 3,180 p.p.m. per unit weight of lignite.

An aliquot of the fatty alcohol fraction was converted to the trimethylsilyl derivatives which were then separated by g.c. as shown in Fig. 49. The chromatogram shows a homologous series of these derivatives ranging from n-C_{20} to nC_{31} with a marked preponderance of the even-numbered members. Identifications were made by coinjecting authentic standard derivatives. Long chain n fatty alcohols are characterisitic constituents of leaf cuticular waxes, thus carnauba wax contains all the even membered alcohols from n-C_{26} to n-C_{34}. Branched-chain fatty alcohols are not present, in substantial amounts, in cuticular waxes. Small amounts of components present between the n-fatty alcohol derivatives in Fig. 49 were not identified.

The isolation of fatty alcohols from German montan wax in the range noted above has been reported previously[186]. Recently, several reports have appeared describing the isolation of fatty alcohols in recent and ancient sediments. However, only small amounts (ca 20 p.p.m.) have been recovered in ancient sediments. Hoering[105], and Sever and Parker[94] have isolated n-fatty alcohols ranging from n-C_{14} to n-C_{26} in extracts of Green River Shale in concentrations of 11 and 20 p.p.m. respectively. The presence of dihydrophytol has been reported by the above authors in this shale. The presence of this compound in lignite has not been

Fig. 49

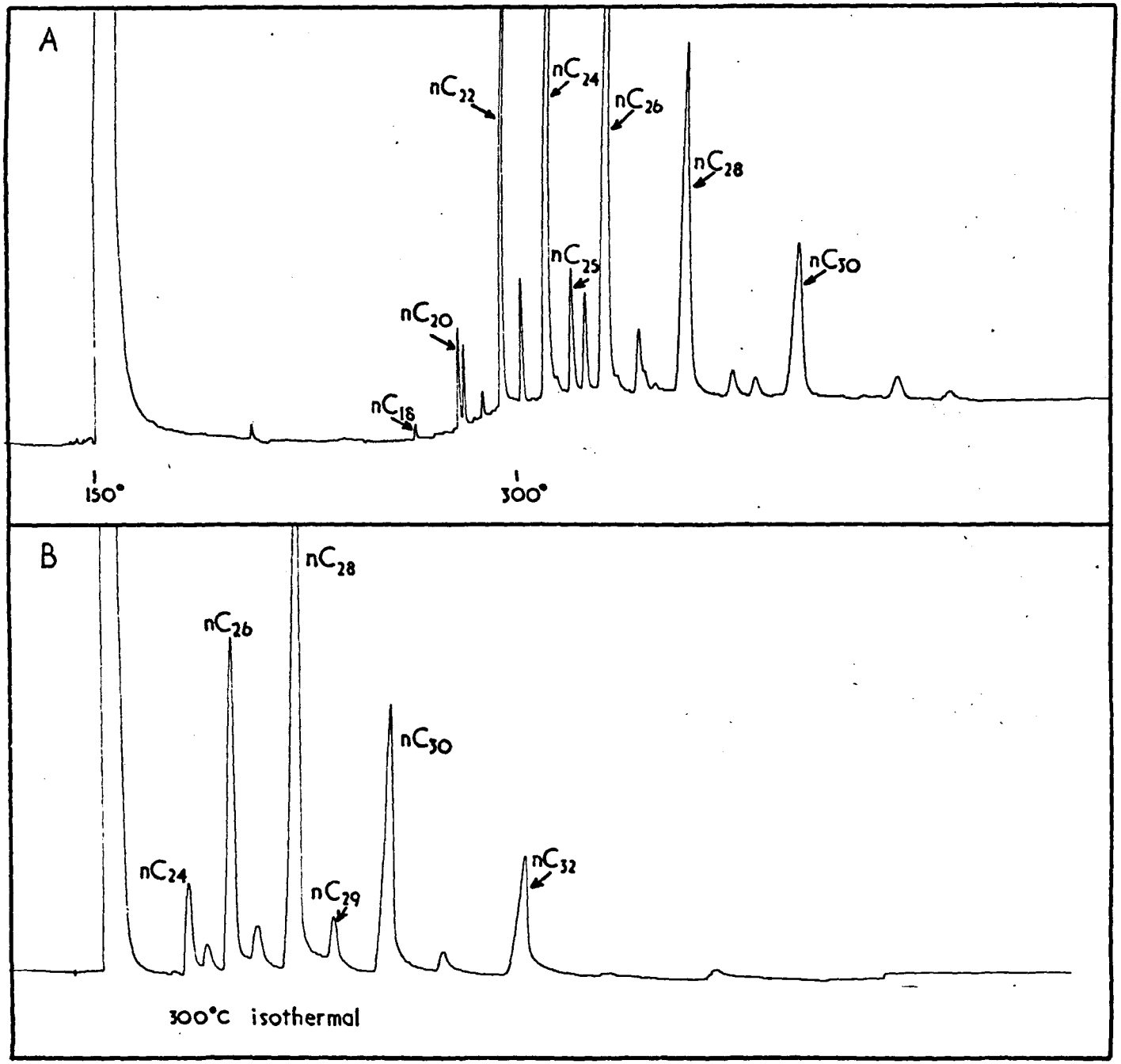
Fatty alcohols (trimethyl silyl derivatives) and fatty acid esters from the Bovey Tracey lignite

A. Fatty alcohol trimethyl silyl derivatives

Chromatographic conditions: as for Fig. 32 except that the oven was programmed between 150° and 300°C at 4°C/min.

B. Fatty acid methyl esters

Chromatographic conditions: as above except that the oven was operated isothermally at 300°C.


A
nC22
nC24
nC26
nC28
nC25
nC30
nC20
nC18
150°
300°
B
nC28
nC26
nC30
nC24
nC29
nC32
300°C isothermal

reported. The retention index of the trimethyl silyl derivative of this compound should be similar to the corresponding derivative of the n-C_{18} alcohol. Visual inspection of the chromatogram in Fig. 49 shows that there is no evidence for its presence in the Bovey Tracey lignite.

Fatty acids in the Bovey Tracey Lignite

The fatty acids which were recovered from the lignite amount to a concentration of 1,900 p.p.m. per unit weight of shale. Fig. 48 shows the chromatogram of the methyl esters and it is evident that a homologous series ranging from at least n-C_{24} to n-C_{32}, is present. These results are similar to those of Hewett et al[137] and Wollrab[183]. It is evident that branched-chain acids are absent and that the distribution is markedly different from fatty acid extracts of ancient sediments such as the Marl Slate and Caithness shales.

Resin fraction components (triterpenoids) in Bovey Tracey Lignite

The resin fraction of the montan wax was chromatographed on alumina (grade 4) using the following elution scheme; petroleum ether, petroleum ether/20% benzene, benzene and benzene/10% methanol. The eluants were monitored by i.r. spectroscopy and combined, appropriately, to give four fractions designated R-1, R-2, R-3 and R-4.

The i.r. spectrum of R-1 showed strong absorption bands at 2930, 2860, 1460 and 1380 cm^{-1}. A strong absorption band at 1708 cm^{-1} and a very weak band at 1770 cm^{-1} suggested a ketone and a five-membered lactone ring respectively. Crystallization of this fraction yielded a white powder (Mpt. 214° - 216°C, $[\alpha]_D^{25}$ = + 51.7°, chloroform). An i.r. spectrum of this compound was similar to that above except that the band at 1770 cm^{-1} had disappeared. Examination of this compound by t.l.c. followed by g.c. suggested that it was more than 98% pure. The retention index of this compound was determined at 310°C, on a high efficiency OV-1 column as 3614 Kovats index units. Its mass spectrum showed a prominent molecular ion at m/e 426 with a base peak at m/e 183 and a mass measurement gave a molecular formula of $C_{30}H_{50}O$. Examination of the literature revealed

that a $C_{30}H_{50}O$ triterpenoid ketone, with the above physical constants has not been reported previously.

The R-2 fraction was crystallized and yielded a white amorphous powder (Mpt 232-235°C, $[\alpha]_D^{25}$ = + 85.0°C chloroform). These data are similar to those for allobetulone, previously identified in this lignite. An i.r. spectrum of this material had the absorption bands noted above, except that the 1775 cm^{-1} band was more prominent. Examination of the material by t.l.c. and g.c. showed that it was impure. Three components were separated by g.c. and their Kovats retention indices were 3423 (57%), 3620 (22%) and 3812 (19%). The figures in parenthesis refer to the relative concentrations of each component. The material showed no tendency to decompose under the g.c. conditions used, since the peaks were symmetrical. The mass spectrum of this mixture showed two molecular ions at m/e 440 and 454, and mass measurement showed that the two compounds had molecular formulae of $C_{30}H_{48}O_2$ and $C_{30}H_{46}O_3$, in agreement with the formulae of allobetulone and oxyallobetulone respectively. It is worth noting that although the physical constants of the crystalline products from R-2 were in agreement with those reported for allobetulone, it was in fact a mixture of three components. The previous isolation and identification of allobetulone by Ikan and McLean[193] was made without the benefit of these newer techniques, and therefore the presence of the associated terpenoids in their samples remains a possibility.

Repeated attempts to crystallise the R-3 fraction were unsuccessful. The i.r. spectrum of this fraction was similar to that of R-2 except that a broad hydroxyl band, of medium intensity was present. Since hydroxyl substituents were indicated, the mixture was converted to its silyl derivative (BSA/ chloroform) and then analysed by g.c. A gas chromatogram of these derivatives showed a major component (ca. 50% of the fraction) with a Kovats retention index of 3854 units. A gas chromatogram of the silyl derivative of the oxyallobetulin standard showed that three components

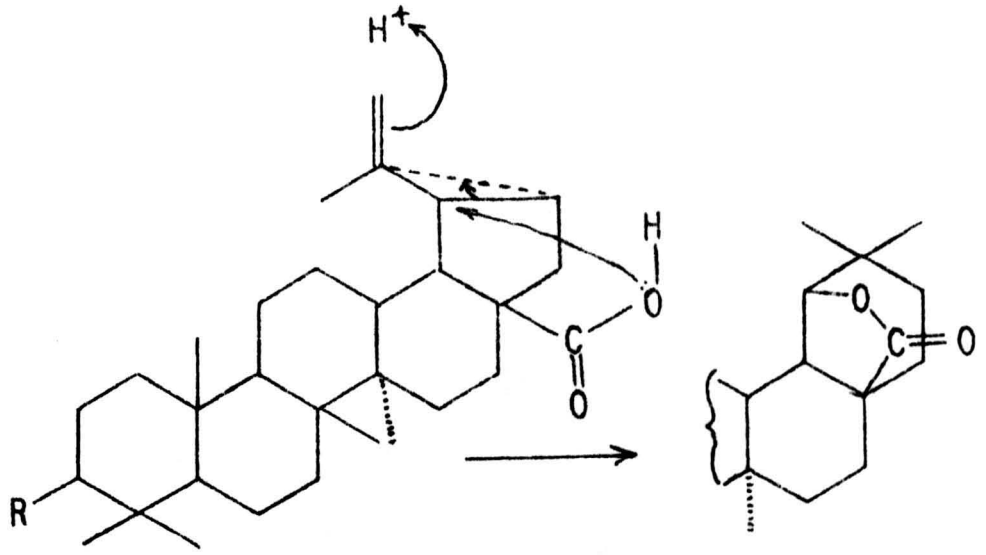
were present, having Kovats retention indices of 3458 (11%), 3657 (27%) and 3852 (60%). From the above data, it is likely therefore that the prominent component of R-3 may be tentatively identified as oxyallobetulin.

The R-4 fraction gave a white crystalline product (Mpt. 263°-266°C). An i.r. spectrum showed strong hydroxyl absorption with no bands at 1710 or 1775 cm^{-1}. T.l.c. indicated the presence of two compounds which had R_f values identical with allobetulin and betulin. After conversion to the silyl derivatives, the retention indices of these components (50% and 46% by weight of the crystalline product), were measured and found to be 3657 and 3600 Kovats units, in excellent agreement with the silyl derivatives of allobetulin and betulin (3658 and 3600 units respectively). A mass spectrum of the crystalline product showed a molecular ion at m/e 442; mass measurement gave a molecular formula of $C_{30}H_{50}O_2$, in agreement with the formula of allobetulin and betulin. T.l.c. and g.c. indicated the presence of a minor component (ca. 4%) in this crystalline product; its silyl derivative had an index of 3458 Kovats units, in excellent agreement with that of the silyl derivative of lupeol which was 3456 Kovats units. On the basis of t.l.c. and g.c. evidence, this minor component may be identified tentatively as lupeol, a triterpenoid that has not been found previously in lignites or sediments.

The retention data (g.c.) for these compounds are shown in table 16, along with similar data for the triterpenoids betulin, allobetulin, oxyallobetulin and lupeol which were kindly provided by Dr. J. McLean, Strathclyde University. In view of the results reported here, it is interesting to postulate the mechanisms by which some of the triterpenoids found in the Bovey Tracey lignite may be derived from naturally occurring triterpenoids.

The triterpenoid lactones occurring in Bovey Tracey lignite and Bohemian brown coals can be related to the triterpenoids containing a lupane skeleton. Two of these triterpenoids, betulonic and betulinic acid, may be converted to their lactones by acid isomerisation involving

a Wagner-Meerwein rearrangement. The reaction, involving protonation of the double bond followed by a ring bond shift and neutralisation of the carbonium ion at the C_{19} position with the lone electron pair of the hydroxyl group, can be written formally as:

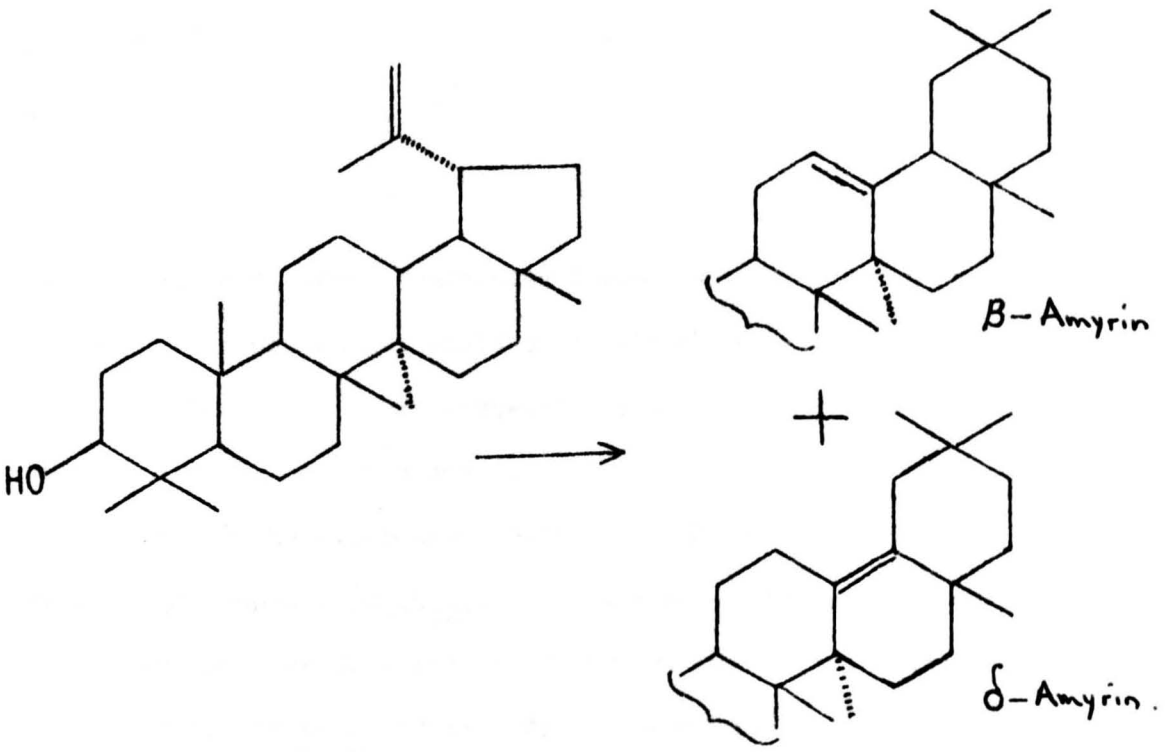


By similar process allobetulin (Fig. 40) present in lignites, could have been derived from betulin (Fig. 39). The formation of these lignites is believed to have occurred in slightly acidic conditions, varying from pH 5.8 to pH 6.1, depending upon the amount of woody tissue present in the decaying rafts of vegetation. This being so, it is possible that the rearrangements discussed above, occur during diagenesis of the lignite.

In the absence of oxygen functional groups at the C_{17} position, as in lupeol, neutralisation of the carbonium ion occurs by hydride shifts yielding β-amyrin and δ-amyrin, as shown below:

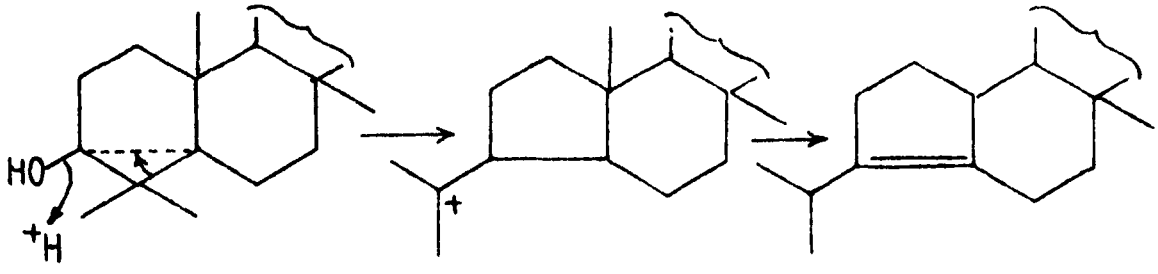
The absence of β - and δ -amyrin in lignites, together with the presence of a number of triterpenoids containing a 3β -OH group, suggests that lupeol has been, at most, a minor constituent of the original plant debris.

This is in agreement with the small amount of lupeol found in this lignite, which has been tentatively identified from its g.c. retention index.



Dehydration involving the 3β -OH group apparently contributes to the diagenetic process. Thus dehydration of oxyallobetulin and allobetulin found in the lignite would give rise to the compounds oxyallobetul-2-ene (Fig. 40) and allobetul-2-ene respectively, reported by Jarolim et al[194,195].

Neutralisation of the carbonium ion at C_3 may occur via a ring bond and hydride shift to yield apoxyallobetulin and α-apoallobetulin from oxyallobetulin and allobetulin respectively. This may be formally written as:



Hydrogenation of these rearranged lupane skeletons and removal of the oxo and ether groups may yield a triterpane with the molecular formula, $C_{30}H_{52}$. Henderson[50] has suggested, from g.c. retention data and mass spectrometry, that a pentacyclic triterpane having this structure may be present in the hydrocarbon extract of Green River Shale. The mass spectrum of hopane, ($C_{30}H_{52}$), is very similar to that of this pentacyclic triterpane but the g.c. retention indices of the two triterpanes are sufficiently different to exclude the possibility of it being hopane.

Friedlan-ol (Fig 39 which occurs in Bohemian brown coals, could, in principle undergo acid isomerisation during diagenesis by a complex 1,2, rearrangement involving 4-methyl and 3-hydride shifts to yield a hydrocarbon with the β-amyrin (oleanane) skeleton, (Hydrogenation and dehydration of δ-amyrin could also produce a saturated pentacyclic triterpane ($C_{30}H_{52}$), β-amyrane (oleanane)). However, strongly acidic conditions are required for this isomerisation, and the absence of amyrin in the Bohemian brown coals suggests that this process has not occurred.

It is interesting to note that hydrogenation of the triterpenoids e.g. apooxyallobetulin and apoallobetulin, may occur by a disproportionation process in which other triterpene skeletons become progressively aromatised. This process has been noted by Jarolim et al[194,195], and it is apparent that the lactone and ether rings are quite stable to reduction, aromatisation of the triterpenoid rings, A,B,C,D, being the more prominent reaction.

Wollrab and Streibl[183] have suggested that the presence of abundant derivatives of betulin in brown coals bears evidence to the fact that the source material is very probably connected with the family Betulaceae. However, the presence of these triterpenoids in the Bovey Tracey lignite can only be explained if they are related to the family Taxodiacea, i.e. the genus Sequoia.

TABLE 13. NORMAL ALKANES IN THE BOVEY TRACEY LIGNITE

CARBON NUMBER	% ABUNDANCE (Calculated from Peak Heights in Gas Chromatogram)
17	0.5
18	0.5
19	1.0
20	0.5
21	1.0
22	1.4
23	7.8
24	4.4
25	13.2
26	6.0
27	18.2
28	7.4
29	15.3
30	5.7
31	12.0
32	2.2
33	2.9

TABLE 14. KOVATS RETENTION INDICES OF BOVEY TRACEY BRANCHED-CYCLIC ALKANES (ON PPE - 7)

PEAK NUMBER*	RETENTION INDEX+ (OV-1)	RETENTION INDEX≠ (PPE-7)
1	2337	2615
2	2377	2676
3		3097
4		3160
5		3168
6		
7		3308
8		3319
9	3139	3357
10		3395
11	3289	3506
12		3530
13		3578
14		3585

* See Fig. 44

\+ Programmed run

≠ Isothermal 250°C

TABLE 15. MOLECULAR FORMULAE OF CYCLIC HYDROCARBONS IN BOVEY TRACEY LIGNITE (cf. Figs. 46 and 47)

SPECTRUM NO.	MOLECULAR FORMULA
A	$C_{24}H_{38}$
B	$C_{24}H_{38}$
C	$C_{24}H_{38}$
	$C_{24}H_{40}$
D	$C_{24}H_{38}$
	$C_{24}H_{40}$
E	$C_{24}H_{38}$
	$C_{24}H_{40}$
F	$C_{24}H_{42}$

TABLE 16. KOVATS RETENTION INDICES OF TRITERPENOIDS ISOLATED FROM BOVEY TRACEY LIGNITE

CRYSTALLINE COMPONENT	RETENTION INDEX (OV-1)
R - 1	3614
R - 2	3423 (major component, 57%)
	3620 (22%)
	3812 (19%)
R - 3 (not crystallised)	3854
R - 4	3657 (major component 50%)
	3600 (46%)
	3458 (4%)
STANDARDS*	
betulin	3600
allobetulin	3658
oxyallobetulin	3852 (60%)
	3657 (27%)
	3458 (11%)
lupeol	3456

* Kindly provided by Dr. J. MacLean, Strathclyde University

CHAPTER IX

CONCLUSIONS

(a) ORGANIC GEOCHEMISTRY OF THE MARL SLATE, KUPFERSCHIEFER AND CAITHNESS SHALES

The four Marl Slate samples from Co. Durham, i.e. the samples from Downhill, Middle Stotfold, Fishburn and the Mainsforth colliery, had organic carbon contents in the range 7.68% (Fishburn sample) to 1.97% (Mainsforth colliery). The lateral equivalents of the Marl Slate in the Nottinghamshire region, i.e. the samples from Kneesall and Milton, contained lesser amounts of organic carbon, 0.41% and 0.68% respectively. This decrease is also seen in the Kupferschiefer samples, K-1 and K-2 which have 0.56% and 0.81% of organic carbon respectively.

Extrac tion of the Marl Slate with organic solvents showed that there is a considerable range in the amount of organic material extracted. Thus, the Co. Durham samples show concentrations in the 5,300 ppm (Middle Stotfold) to 2,870 ppm (Mainsforth Colliery) range, compared with values of 930 ppm (Milton) to 460 ppm (Kneesall) from Nottinghamshire. This variation in organic extracts is more prominent in the Kupferschiefer samples, K-1 and K-2, which show concentrations of 170 ppm and 3,960 ppm respectively. Total alkane concentrations in these samples show a similar decrease towards the Nottinghamshire and North Sea regions. Concentrations vary from 1020 ppm (Fishburn) to 380 ppm (Mainsforth colliery) in Co. Durham, and 32 ppm (Kneesall) to 72 ppm (Milton) in Nottinghamshire, while values for the Kupferschiefer, K-1 and K-2, are 6 ppm and 590 ppm respectively. Gas chromatography of the total alkanes has revealed that a marked similarity exists between the alkane distributions of the Co. Durham and Nottinghamshire samples. Normal alkanes are present in the range $\underline{n}$-C_{13} to $\underline{n}$-C_{30}, with a CPI of about unity (1.04) and an envelope maximum at $\underline{n}$-C_{17} and/or $\underline{n}$-C_{19}, suggesting derivation from marine and lower organisms. Only minor amounts of the

$\underline{n}$-C_{27} and $\underline{n}$-C_{29} alkanes are present suggesting little contribution from terrestrial plants.

A significant change in the distribution of total alkanes is evident when one follows the lateral equivalents of the Marl Slate eastwards under the North Sea, where the sediments have been buried to more than 8,000 feet. Thus, the K-2 sample has an $\underline{n}$-alkane envelope maximum below C_{13} (Fig. 16) and the K-1 samples shows a complex hump of unresolved hydrocarbons, on which are superposed a series of $\underline{n}$-alkanes, in the range $\underline{n}$-C_{15} to $\underline{n}$-C_{24} (Fig. 15).

The C_{13}, C_{14}, C_{15}, C_{16}, C_{18}, C_{19} and C_{20} isoprenoid hydrocarbons have been identified in the Marl Slate by comparing their chromatographic behaviour and mass spectra with those occurring in the Green River Shale and also with authentic isoprenoid hydrocarbons. The gas chromatograms (Figs. 7-10) show that a complex mixture of high molecular weight hydrocarbons are present which are believed to be steranes and triterpanes. Capillary g.c. of the branched-cyclic alkanes, from the Downhill sample, revealed that more than two hundred components were present. Retention data, on 200 ft. capillary columns coated with Apiezon L, were obtained and compared with those of Henderson et al for the cyclic alkanes in the Green River Shale. The retention indices of 5α and 5β cholestane were different to those found by the above authors. Due to this fact, it was not possible to relate the retention indices of unidentified steranes and triterpanes in Marl Slate with those compounds identified in the Green River Shale.

Very few isoprenoid hydrocarbons are evident in the gas chromatograms (Figs. 15 and 16) of the Kupferschiefer samples. Pristane and phytane are present in low concentrations in K-1, but are not present in K-2. These very low concentrations and the absence of steranes and triterpanes suggest that they have been degraded by prolonged burial at elevated temperatures. The results of Eisma and Jurg[90] suggest that the alkanes

may have been degraded to low molecular-weight branched hydrocarbons during their period of burial.

Rayner[13] has reported that marked similarities exist between the Caithness shales and Green River Shale, both in conditions of deposition and the type of precursor organic material, predominantly algal detritus. In comparison to Green River Shale it was found that Marl Slate contained low amounts (6.6 ppm) of fatty acids. A homolgous series of normal alkanoic acids in the range $\underline{n}$-C_{10} to $\underline{n}$-C_{26}, with a slight even/odd predominance and an envelope maximum at $\underline{n}$-C_{16} and $\underline{n}$-C_{18} has been identified. The acyclic isoprenoid acids, C_{13}, C_{14}, C_{15}, C_{16}, C_{18}, C_{19}, C_{20} and C_{21} have also been identified. The distributions of the acids in Marl Slate and Green River Shale are remarkably similar; phytanic and pristanic acids are the predominant acids in both sediments. However, the concentration of total acids in the Marl Slate is two orders of magnitude less than those present in the Green River Shale. Nevertheless, the distinct similarities in these distributions suggest that similar diagenetic processes have altered the precursor organic material present in these depositional environments. Both sediments are rich in saturated alkanes and it appears likely that the low level of fatty acids in the Marl Slate has been the result of decarboxylation reactions, perhaps by a process similar to that postulated by Cooper and Bray[33].

The Caithness samples were unusual in that the organic material extracted from samples A and B, (1400 and 800 ppm) respectively, contained unusually high concentrations of saturated hydrocarbons, namely 63.5% and 22.5%. Sample A, Fig. 20, contains a complex mixture of hydrocarbons which are believed to be the result of degradation of precursor organic material at elevated temperatures. This sediment is close to igneous dykes, and because of the similarities of the hydrocarbon distributions with those in elaterites in Derbyshire it is suggested that this material has been degraded by hydrothermal re-deposition. Sample B which has not been exposed to dykes, shows a much less complex mixture of hydro-

carbons (Fig. 21) with a distribution similar to those in the Marl Slate, except that there is little evidence of hydrocarbons above $\underline{n}$-C_{24}.

The fatty acids that have been isolated from sample B are thought to be the result of post-depositional contamination, *in situ*, at the rock outcrop. The predominant acids are even-membered normal fatty acids, $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$, with substantial amounts of *iso*- and *anteiso*-acids; the latter are indicative of contamination by lower organisms.

The Marl Slate has been shown to contain an unusually high concentration of metalloporphyrins, namely 37.4 ppm of the shale. Nickel and vandium (vanadyl) are the principal chelating atoms. Mass spectra of the metalloporphyrin extracts showed that two structural types were present; MEP and DPEP porphyrins, as illustrated in Figs. 26 and 27. Two series were present, each compound differing in the number and type of alkyl substituents on the pyrrole rings. Following the reports of Baker *et al*[102], it has been possible to determine from DPEP/MEP ratios that the Marl Slate is of marine origin; this is in agreement with geological data[9].

Attempts to synthesise volatile derivatives of porphyrins, suitable for g.c. have not been wholly successful. It is hoped that further work will improve the methods of synthesis or produce more volatile and stable derivatives. Mass spectrometry has proved a valuable technique in the identification of porphyrins, but it is restricted in that it cannot determine the positions of substitution of the alkyl groups on the porphyrin nucleus. Gas chromatography of suitably volatile authentic porphyrin derivatives would most likely help to elucidate the structures of these petroporphyrins. Hydrogenolysis experiments have shown that approximately 67% of the Marl Slate kerogen can be degraded to benzene soluble products. Carbon and hydrogen analyses of the products from hydrogenolysis experiments at 320°C and 280°C have indicated the kerogen is substantially aromatic in character.

Chromatographic data has shown that about 12% of the kerogen is composed of aliphatic moieties. In terms of alkanes this is more than 15 times the amount that can be extracted from the shale. The total, branched-cyclic a and normal alkanes obtained from each of the hydrogenolysis experiments show important qualitative differences. Results of the 320°C hydrogenolysis show that a smooth envelope of normal alkanes is produced (Fig. 32) with a CPI index of almost unity, whereas the experiments conducted at lower temperatures indicate that normal alkane distributions with CPI indices of less than unity are produced, due to high concentrations of the $\underline{n}$-C_{18} and $\underline{n}$-C_{20} alkanes. These alkanes are believed to have been derived from precursor fatty alcohols on acids bound to the kerogen. Fatty alcohols were not found in the Marl Slate and this substantiates the results of previous workers who found very low concentrations only ($<$20 ppm) in much younger sediments.

(b) ORGANIC GEOCHEMISTRY OF THE BOVEY TRACEY LIGNITE

The work reported here represents an extension of that carried out by Ikan and McLean[193].

Extraction of the lignite afforded a montan wax (2.15%) which was separated into various lipid classes. The total alkanes (630 ppm), consist in part of a series of normal alkanes in the range $\underline{n}$-C_{17} to $\underline{n}$-C_{33} with an envelope maximum at $\underline{n}$-C_{27} and $\underline{n}$-C_{29}, and a CPI index of 2.6. Such a distribution is similar to that in contemporary terrestrial plants and it is believed that these alkanes represent the original cuticular waxes present in Sequoia couttsiae.

Branched-cyclic alkanes are present, although no evidence was found for the presence of pristane or phytane, which are of ubiquitous occurrence in ancient sediments. Two tricyclic diterpenes with molecular formulae of $C_{20}H_{34}$ and $C_{20}H_{32}$ have been tentatively identified. These may have been derived from abietic acid, a diterpenoid acid of common occurrence in the order Coniferales, by reduction of the carboxylic

acid group, and reduction of a double bond in the former compound. Nine hydrocarbons were found with molecular formulae of $C_{24}H_{38}$, $C_{24}H_{40}$ and $C_{24}H_{42}$. It is believed that these hydrocarbons have been formed by degradation of original triterpenoids present in Sequoia couttsiae.

The predominant hydrocarbon in the alkane mixture was found to be a homo-triterpane. Mass spectral evidence indicates that the hydrocarbon may be a pentacylic triterpane with a molecular formula of $C_{31}H_{54}$. Although no triterpanes exist in nature, the presence of such compounds containing 31 and 32 carbon atoms has been reported previously in sediments[50], but their structures have not yet been elucidated.

Saponification of the montan wax, yielded a fatty alcohol (3,180 ppm) and a fatty acid (1,900 ppm) fraction. A homologous series of fatty alcohols in the range $\underline{n}$-C_{20} to $\underline{n}$-C_{31}, with a marked preponderance of the even-numbered members was present. A homologous series of fatty acids in the range $\underline{n}$-C_{24} to $\underline{n}$-C_{32} also showed a marked even/odd predominance. Branched-chain alcohols and acids are not prominent, in marked contrast to the occurrence of the latter in ancient sediments. It is believed that these high molecular-weight alcohols and acids represent the original cuticular components of Sequoia couttsiae.

The triterpenoids, betulin, allobetulin, oxyallobetulin, allobetulone, and oxyallobetulone, previously identified by Ikan and McLean[193], have been identified by comparison of their physical

data with authentic compounds. In addition, two other triterpenoids have been found which have not been identified previously in the sedimentary environment. A triterpenoid ketone with molecular formula $C_{30}H_{50}O$, and with physical data that are not in agreement with any reported triterpenoid, has been found. A minor component of the resin fraction has been tentatively identified as lupeol, a triterpenoid that has not been found previously in lignites or sediments.

Finally, processes have been discussed by which the triterpenoids oxyallobetulin and oxyallobetulone could have been derived, by acid isomerisation, from betulinic and betulonic acids respectively. By a similar process allobetulin could have been derived from betulin. The identification of oxyallobetul-2-ene and allobetul-2-ene in Bohemian lignite reported by Jarolim et al[194, 195], may be explained by a diagenetic process involving dehydration of oxyallobetulin and allobetulin respectively.

Although Wollrab and Streibl[183] have suggested that the derivatives of betulin in lignites suggests derivation from plant resins of the family Betulaceae, the presence of these compounds can only be explained if they are related to the genus Sequoia. Thus, before the identity of triterpenoids occurring in lignites

may be used for palaeochemotaxonomic purposes, such identifications should be associated with morphological evidence of the floral content.

CHAPTER X

(a) EXPERIMENTAL DETAILS FOR CHAPTER IV

The Marl Slate, Kupferschiefer and Caithness Shales

(i) Preparation of rock samples

All of the samples were roughly broken with a clean hammer and chisel and the outer surfaces were removed and discarded. In all subsequent handling, polythene gloves, clean tongs and aluminium foil was used. The remaining samples were broken into fragments (ca. 5 cm.) and washed in chloroform ultrasonically, for 10 minutes. Each sample was wrapped in aluminium foil and repeatedly hammered until the average fragment size was ca 2 cm. After a second ultrasonic cleaning, and drying, the samples were pulverised in a disc mill (Tema) in batches of about 150g; the resulting powder from each sample passed 240 mesh. After each operation, the mill was cleaned to prevent cross contamination of samples. The samples were stored in cleaned glass bottles, in an atmosphere of nitrogen, prior to use.

(ii) Extraction of lipids

All samples were soxhlet extracted under similar conditions. Each sample (quantities are recorded in Table 17), was extracted with benzene/methanol (70/30 v.v., 350 ml.) for four days, after which time no colouration of solvent in the siphon was evident. (All solvents, unless otherwise stated, were redistilled through a "60 theoretical plate Oldershaw column"). The solutions were concentrated, under reduced pressure, on a Buchi rotary evaporator and the last traces of solvent were removed at 50°C in a stream of nitrogen to yield viscous oils; yields are recorded in Table 17.

(iii) Column chromatography of the organic extracts

The extracts were chromatographed on alumina (Woelm, Grade 1, sample ratio 50/1), using the following sequence of eluants: petroleum ether, benzene, chloroform and methanol. The only exceptions were for the

TABLE 17. EXTRACTION AND CHROMATOGRAPHIC SEPARATION OF MARL SLATE, KUPFERSCHIEFER AND CAITHNESS SHALES

SAMPLE	SAMPLE WEIGHT g.	ORGANIC EXTRACTS mg.	PET.ETHER ELUATE mg.	BENZENE ELUATE mg.	CHLOROFORM ELUATE mg.	METHANOL ELUATE mg.
MARL SLATE (DOWNHILL)	100	356	52.0	92	180	15.0
DC 322	100	530	96.9	198	143	33.0
BU 4930	100	548	10.2	169	270	70.0
BQ 7276	100	46	3.2	5.2	11.3	4.0
BK 9825	50	93	3.6	6.1	15.6	8.2
YFF 1626	100	287	37.7	88.3	91.0	13.0
K-1 8698 ft.	100	17	.6	present as sulphur		
K-2 10,000 ft.	100	396	59.0	47.0	65.0	215
CAITHNESS SHALES						
Sample A	185	261	165			95.0
Sample B	150	121	27.1			91.0

Caithness shales, where only the first and last eluants were used. Since the extracts were not completely soluble in petroleum ether, the following method was used to charge them to the column. Each extract was heated with petroleum ether (2ml.) and the slurried solution was transferred, with washings, to the chromatographic column. The petroleum ether eluates (total alkanes) were examined by t.l.c., i.r. and g.c., prior to their separation into normal and branched-cyclic fractions.

(iv) Thin layer chromatography of total alkanes

An aliquot of each of the total alkane fractions was analysed by argentaceous t.l.c. on Kieselghel G plates (0.3mm) containing 5% $AgNO_3$. The plates were activated at 110°C for 1 hour and were used immediately after cooling; they were developed with petroleum ether and visualised in u.v. light after spraying with a dilute ethanolic solution of Rhodamine G. All of the samples appeared to be free of olefins.

(v) Infra-red spectrophotometry of eluates

Aliquots of each were analysed as thin films on a Grubb-Parsons spectrophotometer Spectra measured in the range 4,000-650 cm^{-1} have been reported in Chapter IV.

(vi) Gas Chromatography of the alkanes

The total alkanes isolated from the shales (Table 17) were analysed on a Varian gas chromatograph. Stainless steel columns, 20ft x 1/16" O.D. (0.040" I.D.) and 20ft x 1/8" O.D. (0.080" I.D.) packed with 3% OV-1 on Gas Chrom. Q (100/120 mesh) were used. Ovens were programmed at 4°C/minute between 100° and 300/310°C. Nitrogen was used as carrier gas at an inlet pressure of 40-50 psi, equivalent to a flow rate at 100°C of 8ml/minute. Routine tests at 200°C showed that these columns had efficiencies of between 10,000 and 13,000 theoretical plates.

Preparative g.c. was carried out on a Pye 104 machine which was fitted with stainless steel columns, 10ft x 1/4" O.D., packed with 5% SE-30 on Chromosorb W (100/120 mesh). Nitrogen was used as carrier gas

to give a flowrate of 30ml/minute at 220°C. The efficiencies of these columns under these conditions varied between 3,000 and 4,000 theoretical plates. The isoprenoid alkanes from the Marl Slate were collected by splitting the effluent (ca. 10/1 split ratio) and condensing the solutes in glass capillary tubes inserted inside a heated (240°C) stainless steel tube connected to the splitter. The collected isoprenoid alkanes were analysed on an A.E.I. MS9 mass spectrometer at an ionising voltage of 70 ev. and a variable source temperature of 100° - 150°C. We are indebted to Mr. P. Kelly of the Department of Chemistry, for recording the spectra.

(vii) Separation of normal from branched-cyclic alkanes

The total alkane eluates of samples BQ 7276 and BK 9825 were not separated into normal and branched-cyclic alkanes because of insufficient material. Samples K-1 and K-2 were not separated due to the low molecular weights of their components and thus their possible loss by evaporation. Sulphur, present in the total alkanes of K-1, was removed in a column of spongy copper, by Blumer's method.[83]

All other total alkane eluates, were dissolved in anhydrous benzene (5ml), activated 5A molecular sieve (30/1 sieve/sample) was added, and the mixture was boiled under reflux for 24 hours. The supernatant liquid was removed, benzene was added (3ml) and the whole was boiled under reflux for 2 hours. Solvent was removed and the sieve was washed with cold benzene (3 times); the washings were combined with the initial supernatant liquid, and evaporation of the solvent afforded the branched-cyclic alkanes.

The molecular sieve was transferred to a clean polypropylene beaker and distilled water was added (10ml). An aqueous solution of HF (30ml; 40%) was added cautiously to the stirred contents of the beaker and the mixture was left until digestion was complete (ca $\frac{1}{2}$ hour). The gelatinous suspension was neutralised with a saturated solution of

boric acid (30 ml) and then extracted with benzene (2 x 50 ml); the combined extracts were evaporated to yield the normal-alkanes. Both the branched-cyclic and normal-alkanes were filtered separately through short columns of alumina by elution with petroleum ether to yield, after evaporation, purified alkane fractions.

(viii) Preparation of kerogen concentrates

A procedure was developed, essentially from the method of Forsman[145], to remove the inorganic material which enclosed the solvent insoluble organic material i.e. kerogen. This was done so that accurate elemental carbon analyses could be made.

The powdered rock samples (with the exception of the Caithness shales were slowly added with constant stirring, to an excess of concentrated hydrochloric acid (300ml) until effervescence ceased. The contents were allowed to stand overnight and then were filtered under vacuum; distilled water (6 x 30 ml) was added to the filter cake until the filtrates were neutral.

The residues were transferred to polypropylene beakers and stirred to a stiff paste with water (20ml). Concentrated hydrofluoric acid (40%, 200ml) was added gradually and the mixture was stirred and maintained at 60°C for 2 hours. The contents were then allowed to digest for a further 2 hours at room temperature. Excess acid was neutralised with a saturated solution of boric acid (200ml) and the mixture was filtered and washed as before. The procedure was repeated several times until the amount of inorganic material removed was very small. The air dried products were dried in vacuum desiccators containing phosphorous pentoxide.

Elemental carbon analyses of these kerogen concentrates were recorded on an Aminco Carbon/Hydrogen analyser and results are reported in Table 18.

TABLE 18. QUANTITATIVE ISOLATION OF KEROGEN CONCENTRATES IN SHALE SAMPLES

SAMPLE	QUANTITY DISSOLVED g	KEROGEN CONCENTRATE g	% ORGANIC CARBON IN KEROGEN CONCENTRATE
MARL SLATE	300	48.0	42.0
DC 322	95	16.1	26.4
BU 4930	95	23.0	32.0
YFF 1626	95	18.5	10.1
BQ 7276	95	17.7	2.23
BK 9825	48	8.0	4.09
K - 1	100	15.0	3.73
K - 2	100	12.2	6.64

(ix) Isolation of fatty acids

Preliminary experiments showed that fatty acids were not present in detectable amounts, in these shales. Saponification of the solvent extracted kerogen concentrates of the Marl slate and Kupferschiefer showed that detectable amounts of fatty acids were present only in four samples as shown in Table 19. In the Caithness shales, the powdered unextracted shales were saponified and fatty acids were found only in Sample B.

The kerogen concentrates (8-16g) and the Caithness powdered shales (200g) were boiled under reflux with 10% w/w methanolic potassium hydroxide solutions (450ml) for 48 hours. The contents were periodically shaken and stirred to prevent bumping. The resulting mixtures were filtered, the residues were washed with methanol (2 x 50 ml) and the filtrates were transferred to separating funnels. Water (300 ml) was added to each filtrate, which was then extracted with chloroform (3 x 200 ml) to remove unsaponifiable materials. The alternative extracts were acidified to pH 2-3 to liberate the free acids, which were removed by extraction with diethyl ether (3 x 200 ml). The recovered acids (ca 1 mg) were dissolved in a minimum of benzene and applied to t.l.c. plates (0.3 mm thick containing 10% KOH on Kieselgel G). After development with petroleum ether/diethyl ether (95:5), the origins were scraped off, acidified with 1-M. HCl and diluted with water (20 ml). The solutions were extracted with diethyl ether (3 x 20 ml) and evaporated to yield the free acids; amounts are shown in Table 19.

The free acids were methylated with a methanolic solution of boron trifluoride (5 minutes) and the solvents were removed in a stream of nitrogen. The esters were filtered through columns containing alumina (3.0 x 0.3 cm) and eluted with benzene (5 ml), to provide pure ester fractions. These were analysed by t.l.c. and i.r. spectrophotometry (see Section IV).

(x) Thin layer chromatography of the ester fractions

Aliquots of the ester fractions were analysed by t.l.c. using C_{14}-C_{18} fatty acid methyl esters as standards and di-isopropyl ether as developer. Plates were sprayed with concentrated sulphuric acid and charred by heating for 10 minutes (230°C). R_f values were identical and no unsaturated fatty acid esters were detected.

TABLE 19. FATTY ACID CONCENTRATIONS IN THE MARL SLATE AND CAITHNESS SHALE

SAMPLE	CONCENTRATION OF FATTY ACIDS IN ppm OF SHALE
Marl Slate (Downhill)	6.5
BU 4930	6.6
DC 322	0.6
YFF 1626	3.8
Caithness (Sample B)	3.3

(b) EXPERIMENTAL DETAILS FOR CHAPTER V

(xi) Isolation of Marl Slate metalloporphyrins

Marl Slate (145g) was extracted with benzene/methanol (70/30v.v, 350ml) for 50 hours with intermittent stirring. The extracted powder was air dried at 50°C for 1 hour, re-powdered, and slowly added to an excess of concentrated hydrochloric acid (300ml). The mixture was left overnight, filtered, and washed with distilled water (10 x 50 ml) until the filtrate was neutral. The air dried residue (61g) was re-extracted, as above, for a further 50 hours until the solvent in the soxhlet thimble was free from colouration. The dried residue was powdered, added to aqueous hydrofluoric acid (40°C, 50 ml), and magnetically stirred for 4 hours at 60°C. After neutralisation with a saturated solution of boric acid (50 ml), the filtered and washed residue was air dried and stored in a desiccator over activated silica gel.

The residue (23g) was extracted with solvent, as before, until the solvent in the soxhlet extractor was clear.

The extracts were evaporated separately to determine the amount of organic material freed at each stage in the procedure; yields are shown in Table 20.

TABLE 20. YIELDS OF ORGANIC MATERIAL FROM MARL SLATE

	Extract	Yield of organic material g.
(a)	Initial extract	1.28
(b)	Extract after HCl. treatment	0.42
(c)	Extract after HF treatment	0.32

(xii) Column chromatography of the organic extracts (a) and (b)

(1.70g, Table 19), were boiled with petroleum ether (2 x 5 ml) and the cooled solution was chromatographed on alumina (alumina/sample ratio = 30 1), using the elution scheme shown in Table 21. Extract (c) was similarly analysed. Each fraction was concentrated, transferred to a small vial and evaporated to dryness in a stream of nitrogen.

TABLE 21. CHROMATOGRAPHY OF EXTRACTS ON ALUMINA

Fraction No.	Eluant (150ml)	Extract (a) and (b) mg.	Extract (c) mg.
1	Petroleum ether	76	0.5
2	Petroleum ether - 10% benzene	83	0.5
3	Benzene	58	1.0
4	Benzene - 10% methanol	380	280
5	Methanol	26	16

Each fraction was analysed by visible light spectrophotometry (325-700 mμ) and it was established that porphyrin-containing material, identified by a Soret absorption band near 400 mμ, was present only in fraction 4 (benzene-10% methanol fraction) of extracts (a) and (b). This fraction 380mg) was further separated into several metalloporphyrin fractions by chromatography on silica gel. Thus, a petroleum ether extract (2 x 5 ml)

of fraction 4 was chromatographed on silica gel (12g previously activated at 120°C for ½ hour), using the elution scheme shown in Table 22. Each of the six fractions was concentrated in small vials as described above.

TABLE 22. CHROMATOGRAPHY OF FRACTION 4 ON SILICA GEL

Eluant (150ml)	Yield mg.	Fraction no.
Petroleum ether - 10% benzene	18.00	1
Petroleum ether - 50% benzene	9.40	2
Benzene - 50% chloroform	26.20	3
Chloroform	18.40	4
Chloroform - 10% methanol	70.00	5
Methanol	116.40	6

(xiii) Identification of Marls Slate metalloporphyrins by visible light spectrophotometry

The identity of the chelating metals in the metalloporphyrins present in the six chromatographic fractions (Table 22) was determined by visible light spectrophotometry on a Unicam SP800 machine. Quantitative determinations of the metalloporphyrins in each fraction were calculated from the Beer and Lambert relationship, $\mathcal{E} = A/c.l.$, where $\mathcal{E}$, A, c and l represent the molar extinction coefficient, absorbance, concentration (g. mol./l) and path length (cm.) respectively. The molar extinction coefficient was taken as 3.3×10^5 l. mol.$^{-1}$. The procedure was essentially that of Hodgson et al.[113]

Aliquots of each fraction (ca. 1 mg) were weighed in aluminium boats on a Cahn Electrobalance (weighings were accurate to 10^{-3} mg.). A boat and sample was transferred to a gravimetric flask (10 ml) and chloroform (10.0 ml) was added. The absorbance values at 393 mμ (λmax. Ni - porphyrin) and 408 mμ (λ max. VO - porphyrin) were obtained for these fractions and the concentration of metalloporphyrins in each of the six fractions was calculated.

Concentrations are recorded in Table 23 along with the identity of the chelating metal in each of the fractions. These metalloporphyrins

were analysed further by t.l.c. (see Section xx).

TABLE 23. CHELATING METALS AND METALLOPORPHYRIN CONTENT OF THE MARL SLATE

Fraction No.	Metalloporphyrin content mg.	Chelating atoms	Concentration p.p.m. of shale
1	0.80	Ni	5.5
.	0.69	M (unknown)	4.7
2	0.53	Ni	3.5
3	1.49	VO	10.3
4	0.78	VO	5.4
5	1.26	VO	8.0
6	No trace	—	—

(xiv) Demetallation of Marl Slate metalloporphyrins by a modified Groennings method

A crude metalloporphyrin extract (obtained by method xii, 250 mg), was transferred to a carius tube 15 ml and HBr in acetic acid (47%, 5ml) was added. The tube was purged with nitrogen, sealed and heated at 60°C for 5 days with intermittent shaking. The reaction mixture was diluted with water (50 ml), neutralised with 1M NaOH, and extracted with chloroform (3 x 30 ml). The combined extracts were evaporated to yield the free porphyrins (227 mg).

(xv) Demetallation of Marl Slate metalloporphyrins by the Erdman method

A metalloporphyrin extract (250 mg) was dissolved in methanesulphonic acid (3 ml) and the tube was sealed, shaken and heated at 50°C for 1 hour. The reaction mixture was diluted with water (100 ml), neutralised as above, extracted with methylene dichloride (3 x 30 ml). The combined extracts were evaporated to yield the porphyrins (213 mg).

Visible spectra of the products of this demetallation and that above (xiv), were recorded in chloroform solution. It was noted that procedure (xv) was quantitative, since no bands absorbing at λmax. 573,

553, 533 and 516 mμ, indicative of Ni and VO-metalloporphyrins were present. The modified Groennings procedure was not quantitative.

(xvi) Synthesis of Etioporphyrin I (A.W. Johnson, unpublished results)

(a) Preparation of 3-ethylpentane-2,4,-dione.

Ethyl iodide (155 ml), acetylacetone (175 ml) and potassium carbonate (220g) were heated under reflux overnight. The product was filtered and the residue was washed with acetone (4 x 250 ml). Acetone was removed by evaporation and the remaining oil was distilled to yield 3-ethypentane - 2,4-dione (140g), (Bpt. 178° - 181°C).

(b) Nitrosation of tert. - butyl acetoacetate.

Tert. - butyl acetoacetate (200g) was dissolved in glacial acetic acid (213 ml), cooled to 0°C, and a solution of sodium nitrate (90g) in water (155 ml) was slowly added over a period of 2 to 3 hours. The temperature was maintained at 0° to 10°C during this addition and the solution was left overnight at 5°C.

(c) Preparation of tert.-.butyl-2,4,-dimethyl-3 ethylpyrrole-5-carboxylate.

charboxylate

The nitrosated tert.-butyl acetoacetate was added slowly to a stirred solution of ethyl pentanedione (135g) in glacial acetic acid (177 ml), containing zinc dust (110g), at such a rate that the temperature was maintained at 65-70°C. After 2 hours the mixture was poured into water and the crystalline product was filtered off and recrystallised from ethanol (1.5l) to yield 75g of product.

(d) Preparation of a mixture of bromodipyrromethenehydrobromides

Tert.-butyl-2,4-dimethyl-3-ethylpyrrole-5-carboxylate (19g) was dissolved in acetic acid (156 ml) and a solution of bromine (12.5 ml) in glacial acetic acid (62 ml) was slowly added with stirring. After 2 hours at ambient temperature, the product was filtered, washed with petroleum ether (50 ml) and dried in a desiccator over sodium hydroxide pellets. This procedure was repeated three times giving a total yield of 50g (metallic blue crystals).

The mixture (50g) and succinic acid (131g) were ground and fused at 220°C for 1 hour. The flask was broken and the solidified melt was thoroughly ground, suspended in a solution of sodium hydroxide (156g) in water (1.25l), and stirred on a water bath for $3\frac{1}{2}$ hours. After filtration, the residue was washed with water until the washings were neutral and then with methanol; it was then continuously extracted (soxhlet extractor) with methanol for 16 hours. This extract was discarded. The residue was then extracted with chloroform for 4 days, the chloroform solution was concentrated and etioporphyrin-I- was crystallised as purple acicular crystals (5.4g).

(xvii) Preparation of bis-(trimethylsiloxy) - silicon (IV) - etioporphyrin - I

Triethylamine (4.0ml) was added to etioporphyrin I (70 mg) in a Carius tube (15 ml). The solution was cooled to -20°C and silicon tetrachloride (0.8 ml) was added very slowly and with caution. The tube was purged with nitrogen, sealed and shaken in an oven at 170°C for 8 hours. Excess silicon tetrachloride was removed in a stream of nitrogen and the residue was extracted with chloroform (2 x 10 ml). After hydrolysis of the extract with ethanol (2 ml) in 1M hydrochloric acid (10 ml), the solution was evaporated and re-extracted with chloroform (4 x 10 ml). The extracts were combined, evaporated (to 2 ml) and chromatographed on alumina (Grade II, 5g) using chloroform - 10% methanol as eluant (50 ml). The solvent was evaporated to yield dihydroxy-silicon (IV)-etioporphyrin - I (52 mg). The product was analysed by t.l.c., u.v. spectrophotometry and mass spectrometry; the physical data were in good agreement with previously published results[97].

The dihydroxy compound (50 mg) was dissolved in pyridine (2 ml), bis(trimethylsilyl) acetamide (BSA) was added (0.5 ml) and the solution was heated to 90°C overnight. After evaporation of the solvents the product was analysed by t.l.c. using benzene/chloroform (90/10 v.v.) as developer. Bands with R_f values of 0.70 and 0.26 were scraped off,

extracted with chloroform, and evaporated to yield 4.0 and 20 mg. respectively. These components were analysed by m.s. and were shown to be bis(trimethylsiloxy-silicon$^{(IV)}$-etioporphyrin-I and dihydroxy-silicon$^{(IV)}$-etioporphyrin I (see Table 22). Material (168 mg) with an R_f value intermediate to these two bands was shown by m.s. to be a monosilylated product.

(xviii) Preparation of bis-(trimethylsiloxy)-silicon$^{(IV)}$ derivatives Marl Slate porphyrins

A Marl Slate porphyrin-containing fraction (216 mg) was treated as above to yield dihydroxy silicon$^{(IV)}$ derivatives (186 mg). Visible light spectrophotometry showed three absorption bands, characteristic of chelated porphyrins, with absorption maxima at; λ max $^{CHCl}3$ 405, 538 and 578 mμ. Silylation of the dihydroxy derivatives was carried out as above and the products were analysed by t.l.c. (see (xx)).

(xix) Preparation of titanyl etioporphyrin-I

Etioporphyrin I (30 mg) was dissolved in anhydrous pyridine (3.0 ml) in a Carius tube. After cooling to -5°C, titanium tetrachloride (0.5 ml) was slowly added and the tube was sealed and heated at 180°C for 4 hours.

Extraction of the reactants with chloroform (20 ml), followed by hydrolysis with an ethanolic solution (2 ml) of 1 M hydrochloric acid (10 ml) gave, after evaporation, a residue which was further extracted with chloroform (4 x 10ml). After concentration of the extract to 2 ml, the solution was purified by chromatography on alumina (grade II, 5g) using chloroform-10% methanol as solvent. Removal of the solvent yielded a product (24 mg). Visible light spectrophotometry showed absorption bands at λ max $^{CHCl}3$ 405,535 and 574 mμ, which was characteristic of a chelated porphyrin. A mass spectrum of this product showed a prominent molecular ion at m/e 540 corresponding to the presence of a titanyl group (Ti = O) chelated with the etioporphyrin - I nucleus.

(xx) Thin layer chromatography of the porphyrin extracts

Plates were spread with Kieselgel G to give thicknesses of 0.25 or

0.30 mm. Plates were activated at 110°C for 1 hour prior to use and benzene/chloroform (90/10 v.v.) was used as developer. Spots were located by their colour and their fluorescence under u.v. light. Free porphyrins showed intense fluorescence in contrast to the subdued fluorescence which is characteristic of chelated porphyrins. R_f values of the porphyrins which were examined are shown in Table 24. This technique was used to ascertain if the procedures, detailed in sections (xiii) to (xx) were successful.

TABLE 24. THIN LAYER CHROMATOGRAPHIC SEPARATIONS OF THE PORPHYRINS DESCRIBED IN SECTIONS (xiii) to (xx).

Porphyrin	R_f (benzene/chloroform 90/10 v.v.)	Colour	Fluorescence Colour
Etioporphyrin-I	0.46	red-purple	intense red
Nickel porphyrins (Marl Slate)	0.65	red-pink	subdued red
Vanadyl porphyrins (Marl Slate)	0.31	red	subdued red
Dihydroxy silicon$^{(IV)}$-etioporphyrin-I	0.26	red	orange
Bis-(trimethylsiloxy)-silicon$^{(IV)}$ etioporphyrin-I	0.70	red	orange
Dihydroxy silicon$^{(IV)}$ porphyrins (Marl Slate)	0.28	red	orange
Bis-(trimethylsiloxy)-silicon$^{(IV)}$ porphyrins (Marl Slate)	0.72	red	orange

(xxi) Gas chromatography of bis-(trimethyl siloxy)-silicon$^{(IV)}$ etioporphyrin-I

Analyses were carried out on glass columns (3ft x 1/4" O.D.) containing Corning glass beads (80/100 mesh) coated with a 1% solution of a silicone gum, OV-1, in chloroform. After conditioning at 270°C in a Pye 104 instrument, the column was operated isothermally at temperatures of 200° and 270°C using a nitrogen flow rate of 15 ml/minute. Prior to analyses, BSA (10 µl) was injected followed by the porphyrin derivative dissolved in chloroform. Repeated injections

of 1 µl each were necessary before components began to elute from the column. Examination of the column packing, in situ, under u.v. light revealed a strong orange fluorescence, showing that adsorption of the porphyrins had occurred, probably with any remaining free hydroxyl substituent present on the glass surface.

(c) EXPERIMENTAL DETAILS FOR CHAPTER VI

(xxii) Hydrogenolysis of Marl Slate Kerogen

Marl Slate kerogen prepared as previously noted (see viii), was used for these hydrogenolysis studies, which were carried out in a stainless steel vessel (500 ml) at a hydrogen pressure of 110 atmospheres (1,650 psi). After charging the kerogen samples to the vessel it was flushed with hydrogen to remove air, sealed, and quickly heated to the required temperature. This temperature was maintained using a manually operated variable pheostat.

(xxiii) Hydrogenolysis at 320°C for 2 hours

Kerogen (20g) and stannous chloride (8g) were thoroughly mixed together into a homogenous powder. The mixture was placed in a small steel crucible, sealed within the hydrogenator and then heated at 320°C for 2 hours. After cooling, the contents were extracted with benzene (soxhlet) to yield a soluble fraction of 8.2g. Analysis gave C, 72,5% and H, 8.6%.

This fraction (8.1g) was extracted with petroleum ether (2 x 20 ml.), and the combined extracts were evaporated and chromatographed on alumina (50g). The petroleum ether eluate from this chromatogram (250 ml) was evaporated to yield a clear oil (1.09g). The recovered alkanes were then separated into a normal (8.5 mg) and branched-cyclic (712 mg) fraction, using the 5A molecular sieving technique described previously (see vii).

(xxiv) Hydrogenolysis at 280°C for 2 hours

A mixture of kerogen and stannous chloride was prepared and heated

as above, except that a temperature of 280°C was used. Benzene extraction of the product yielded (2.96 g) of soluble material, analysis of which gave C, 73.2%, H, 7.3%. Extraction of this material with petroleum ether, followed by chromatography of the concentrated extract on alumina (20g), yielded a clear oil (186 mg) from the petroleum ether eluate. The recovered alkanes were then separated into a normal (25 mg) and a branched-cyclic (130 mg) fraction by the 5A molecular sieve technique.

(xxv) Hydrogenolysis at 200°C for 64 hours

A mixture of kerogen and stannous chloride, prepared as above, was heated at 200°C for 64 hours. Benzene extraction of the reaction mixture afforded 2.84 g. of soluble material from which a hydrocarbon fraction was obtained, as a clear oil (118 mg) by chromatography on alumina (20g), as above. The oil was separated into a normal (12.4 mg) and a branched cyclic (81.2 mg) fraction by the 5A molecular sieve technique.

(xxvi) Hydrogenolysis at 175°C for 115 hours

Marl Slate kerogen (17g) and stannous chloride (6.8g)were heated at 175°C for 115 hours. Extraction of the benzene soluble material (755 mg) with petroleum ether (2 x 10 ml) afforded an oil which was chromatographed on alumina (12g). This petroleum ether eluate (200 ml) gave a total alkane fraction (60 mg), which from both i.r. and t.l.c. evidence, was found to contain unsaturated hydrocarbons.

The total alkanes (58 mg) were separated by argentaceous t.l.c. into two bands, using petroleum ether as developer. The bands (R_f = 0.91 and 0.50) were removed and extracted with chloroform to yield two fractions.

(a) R_f = 0.91 (47.2 mg) and

(b) R_f = 0.50 (9.0 mg)

Fraction (a), which contained saturated hydrocarbons, was separated into a normal (5.5 mg) and a branched-cyclic (36.1 mg) alkane fraction by the 5A molecular sieve technique.

The total branched-cyclic and normal alkanes isolated from the above experiments, were analysed by g.c. using conditions discussed previously

(see vi). The only exception was that column ovens were programmed at 2°C per minute.

(d) EXPERIMENTAL DETAILS FOR CHAPTER VIII

(xxvii) Extraction of lipids from Bovey Tracey lignite

The lignite (200g) was powdered and extracted for 3 days with benzene (400 ml) in a sohxlet apparatus. Evaporation of the solvent yielded a montan wax (4.3g) which was then refluxed with isopropanol (50 ml) and methanol (50 ml) for 30 minutes. The hot supernatant liquid was decanted to leave a residue which was again extracted with this solvent mixture (50 ml). The supernatant liquids were combined and allowed to cool. The residue (0.99 g) represented the asphalt fraction.

The supernatant liquid was chilled to -5°C and filtered. The precipitated wax was washed with chilled methanol (4 x 15 ml) to yield the wax fraction (2.1 g). The filtrate, and washings, were evaporated to yield the resin fraction (1.04g).

(xxviii) Saponificat..on of the wax fraction

The wax (2.1g) in benzene (20 ml) was boiled under reflux with a methanolic solution of 1M potassium hydroxide (10 ml) for 5 hours, Solvent was removed and benzene (50 ml) was added to dissolve the wax. The solution was chilled to -5°C and the precipitated potassium salts were filtered and washed with chilled benzene (2 x 10 ml). The filtrate and washings were evaporated to yield unsaponifiable lipids (1.37g). The precipitate was acidified to pH=3 with 1M hydrochloric acid, water (20 ml) was added and the whole was extracted with dethyl ether (2 x 30 ml). The extracts were combined, evaporated and methylated (see section ix) to yield a fatty acid methyl ester fraction (38 mg). Infra-red examination of the extracted fatty acids showed a strong carbonyl band at 1700cm^{-1}. with alkane absorption bands at 2960, 2840, 1460, 1376, 726-716 (doublet)cm^{-1}. After methylation, the absorption band at 1700cm^{-1} was shifted to 1744cm^{-1}.

(xxix) Chromatography of the unsaponifiable lipids

The unsaponifiable lipids (1.37g) slurried in petroleum ether (2 ml) were chromatographed on alumina (40g) using the following elution scheme; petroleum ether (250 ml), benzene, benzene-10% methanol (200 ml each). The eluants were concentrated and their weights are recorded in Table 12.

The petroleum ether eluate (126 mg) was separated with 5A molecular sieve (see section vii) into a normal (54 mg) and a branched-cyclic (57 mg) alkane fraction. Analysis by t.l.c. and i.r. indicated that alkanes were absent.

The benzene-methanol eluate (680 mg) was chromatographed again on alumina (20g) using the elution scheme shown in Table 12 (using 50 ml each of solvent). After evaporation, a fatty alcohol fraction (635 mg) was obtained which when analysed by t.l.c. and i.r. was shown to contain no unsaturated alcohols. The alcohols were analysed by g.c. as their trimethylsilyl derivatives, using the conditions described in section (vi). These derivatives were prepared by dissolving an aliquot (80 mg) of the fatty alcohol fraction in chloroform (2 ml), adding trimethylchlorosilane (0.5 ml), hexamethyldisilazane (0.1 ml), and heating for 2 hours at 50°C. This solution was then used for g.c. analysis. Homologues were identified by coinjection of the trimethylsilyl derivatives of n-tetracosanol and n-hexacosoanol.

(xxx) Chromatography of the resin fraction

The resin fraction (950 mg) was chromatographed on alumina (Grade II, 30g), using the elution scheme (100 ml each solvent) shown in Table 12. The eluates, which varied in colour from light yellow to brown, were evaporated to yield the amounts which are reported in Table 12. Each of the resulting oils was triturated with petroleum ether until crystallisation had started, and than left for a period of several weeks at -20°C. The crystalline products were recrystallised from the same solvent and were designated R-1, R-2 and R-4. No crystalline product was obtained for the benzene eluate R-3. The crystalline products, R-1,

R-2 and R-4 were examined by t.l.c., i.r., g.c. and m.s.

The petroleum ether eluate yielded a crystalline compound (21 mg, Mpt. 214°-216°C, $[\alpha]_D^{25}$ =+51.7° (chloroform)). An i.r. spectrum showed alkane absorption bands at 2930 to 2860, 1460 and 1380 cm^{-1}. A strong band at 1708 cm^{-1} indicated a carbonyl group. Examination by t.l.c. on Kieselgel G plates, using di-isopropyl as developer, suggested that only one component was present. Plates were sprayed with a 50/50 mixture (10 ml) of acetic anhydride and concentrated sulphuric acid in methanol (50 ml) and were heated at 75°C for 10 to 15 minutes. (Liebermann Burchard reaction). Analysis by g.c. (isothermal 310°C; see section vi) suggested that the compound was at leat 98% pure. A mass spectrum of this compound showed a prominent molecular ion at m/e 426, and mass measurement indicated that the molecular formula was $C_{30}H_{50}O$.

The petroleum ether - 20% benzene eluate yielded a crystalline compound (40 mg) which from its mleting point (232-234°) appeared to be pure; its rotation was measured as $[\alpha]_D^{25}$ =+85.0° (chloroform). An i.r. spectrum showed the above absorption bands plus an additional band of medium intensity at 1770 cm^{-1}, indicative of a five membered lactone ring. However, t.l.c. indicated that three components were present and this was confirmed by g.c. A mass spectrum of this mixture showed two molecular ions at m/e 440 and 454 and mass measurement showed that these two components have molecular formulae of $C_{30}H_{48}O_2$ and $C_{30}H_{46}O_3$.

The benzene eluate did not crystallise. An i.r. spectrum of the oil was similar to the above except that a broad band at 3360 cm^{-1}, indicative of hydroxyl substitution, was present. Gas chromatographic analysis of the silyl derivatives (see section xxix) showed that it consisted of three prominent components.

The benzene-10% methanol eluate yielded a crystalline component (m.pt. 263°-266°C, change of crystal form at 220°C). An i.r. spectrum showed a strong band at 3370 cm^{-1} and one of medium intensity at 1770 cm^{-1}. T.l.c. showed it was a mixture of two components with R_f values identical

to those of betulin and allobetulin. G.c. of the silyl derivatives showed them to have identical retention indices to the silyl derivatives of the named, authentic compounds.

A mass spectrum of this mixture showed a molecular ion at m/e 442 with a prominent (M-18) fragment due to loss of water. Mass measurement gave a molecular formula of $C_{30}H_{50}O_2$ in agreement with that of allobetulin and betulin.

The methanol eluate yielded an oil (7.0 mg) from which no crystals were obtained; this fraction was not investigated further.

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